ENVIRONMENTAL EFFECTS ON MATE CHOICE DYNAMICS IN THE ZEBRA FINCH (*TAENIOPYGIA GUTTATA*)

Michael Owen Fisher

Presented for the degree of Doctor of Philosophy

Division of Environmental & Evolutionary Biology

Institute of Biomedical and Life Sciences

University of Glasgow

August 2005

Candidate's declaration

I declare that the work recorded in this thesis is entirely my own unless otherwise stated, and that it is of my own composition. No part of this thesis has been submitted for any other degree.

Michael Fisher

August 2005

Acknowledgements

I thank Pat Monaghan and Ruedi Nager for being superb supervisors throughout my PhD work. They provided encouragement, guidance and a wealth of insight. The Natural Environment Research Council funded this work and I am also very grateful to them for allowing me this opportunity.

John Laurie, Alistair Kirk, Graham Adam, Dorothy Armstrong, Helcia Lepitak and June Freel fed the zebra finches and maintained their welfare. Their help was invaluable. Godefroy Devevey provided assistance with some of the mate preference tests. Jon Blount, Tim Harvey and Aileen Adam helped with the antioxidant assays. Kate Orr and Aileen Adam sexed some of my nestlings. Geoff Baxter and his team in the mechanical workshop constructed the apparatus that the birds used during the associative learning task. Nozrat Mirzai and others in the electronic workshop helped resolve technical problems involving stubborn cameras and recalcitrant circuitry. A very special thanks to Mike McMahon, who allowed me access to his phonetics lab and talked me through the use and interpretation of sonograms.

I thank the friends I have met during my time in Glasgow, in particular Diane Baum, Colin Beale, Maria Bogdanova, Liliana D'Alba, Meg Dickens, Lotta Djupsund, Steve Ewing, Chris Foote, Chris Fraser, Helen Gorman, Andrew Jackson, Sin-Yeon Kim, Jo Mould, Veronica Neves, Clare Stamper, Kong Tharapoom, Craig Walling and Patrick Walsh and all the Tuesday football crowd. I also thank my various flatmates, Frank Dewotor, Zara Neumann, Jay Richardson and Ryan Shand, for their friendship. My sister, Helen, I thank for still visiting me despite this necessitating a long trek. And of course, I thank my parents for all their support and love.

Contents

List of figures

List of tables

Abstract

Several aspects of the mating preferences of male and female zebra finches, *Taeniopygia guttata*, were examined. In particular, the role of the dominance status and breeding competence in mate selection was studied. Factors that may influence the preferences of pair-bonded birds, such as relative breeding success and relative nutritional condition, were investigated. In addition, experiments were conducted that looked at the effects of neonatal nutritional environment on adult traits that have been linked with mate choice.

In many cases, mating preferences were dependent on the choosiness (i.e. effort invested in mate assessment) and status (i.e. age and prior experience) of birds. Only the choosiest males preferred competently breeding females, and these males also tended to prefer relatively heavy mates and mates that had received high-quality neonatal nutrition. Older, more experienced males tended to prefer females that were relatively heavy, possessed intermediate bill colours and were from a high-quality nutritional background. Similarly, relatively old, experienced females preferred competent breeders rather than naïve males.

Zebra finch pair bonds were generally strong; the majority of birds affiliated more with their established partner than they did with alternative mating options, regardless of prior breeding success. The strength of female preference for an established partner, however, was reduced by reproductive failure. Bond stability was also higher among pairs in which birds were relatively heavy, males were relatively old and females were relatively young.

The impact of a neonatal nutritional deficit on adult morphology was dependent on the severity of the nutritional restriction. Generally, birds that experienced a deficit exhibited a varying degree of compensatory growth. Antioxidant capacity, which is thought to determine the expression of male secondary sexual traits, was highest among individuals aged 4 months that had experienced fast neonatal growth, rather than among those that showed slow early growth and then compensated. However, among birds aged approximately 17 months, the highest antioxidant capacities were found among birds that received low-quality neonatal nutrition but compensated little subsequently.

Male song learning was impaired by neonatal nutritional deficit, which is consistent with previous work. In contrast, a poor developmental nutritional

environment itself produced no adverse effect on adult performance on an associative learning task. Associative learning performance was worst, however, in those individuals that grew relatively fast, and thereby compensated, subsequent to a nutritional deficit.

Chapter 1

General introduction

Mate discrimination

Animals should discriminate between mates only when these mating options vary in their value to the selecting individual (Parker 1983; McNamara *et al.* 2003). The value of a mate (i.e. mate "quality") is determined by its genetic quality, the resources it offers or its parental abilities. Mating preferences (i.e. the sensory and behavioural properties that influence the tendency of individuals to mate with certain phenotypes: Heisler *et al.* 1987) can evolve for traits that indicate value (Kirkpatrick & Ryan 1991). The term "mating preferences" actually comprises two concepts (Jennions & Petrie 1997): (1) preference function (i.e. the order in which prospective mates are ranked based on particular traits) and (2) choosiness (i.e. the effort invested in assessing mates). Furthermore, mating preferences and "resistance" to mates, can together be considered examples of "mating biases" (Kokko *et al.* 2003). The mating pattern that emerges within a population is a result of actual "mate choices" that stem, in part, from the expression of these mating biases (Jennions & Petrie 1997; Kokko *et al.* 2003).

In most species, males compete over mates to a greater extent than females. Females generally exhibit higher parental investment (PI) (i.e. parental behaviour or characteristics that increase offspring fitness at the expense of future parental fitness, *sensu* Trivers 1972) and they therefore possess a lower potential reproductive rate (PRR) (Clutton-Brock & Vincent 1991) than males. Females consequently act as a limiting resource over which males tend to compete (Clutton-Brock & Vincent 1991). Sex differences in choosiness are less easily explained; choosiness and competition are not opposite alternatives (Kokko & Monaghan 2001). On the one hand, the sex with the highest PI is likely to show a greater variation in parental quality. This may lead us to expect that the sex investing less will be more discriminating as they stand to benefit more from choice (Parker 1983; Owens & Thompson 1994). On the other hand, the costs

of mate choice, in terms of the time required to locate alternative mates, is expected to be higher for the sex with the lower PI and higher PRR. Therefore, conversely, this sex might be expected to be less choosy (Parker 1983; Johnstone *et al.* 1996). Recent models have suggested that it is the cost of breeding, which is tantamount to Triversian PI, which is the most important determinant of choosiness (Kokko & Monaghan 2001; Kokko & Johnstone 2002). The sex with the higher costs associated with a single breeding attempt, which on the whole is females, should be the choosiest.

In polygamous breeding systems such as leks, in which only one sex provides parental care, making predictions about sex roles is thus a comparatively straightforward exercise. Although females are usually the choosy sex, some species exhibit sex-role reversal (e.g. polyandrous breeding systems: Emlen & Oring 1977), which entails that males are more selective than females. In some cases, however, the initial selection of a mate can result in the formation of a socially monogamous partnership in which both individuals provide parental care (Black 1996). In these species, it is not usually a simple matter of one sex being choosy and one sex being competitive. Various combinations of choosiness in the two sexes can occur, depending on the costs and benefits that each sex accrues from being selective (Owens & Thompson 1994; Johnstone *et al.* 1996). In some of these biparental care species, the sexes have comparable levels of parental investment and thereby bear similar costs of breeding. Males, as well as females, are likely to be discriminating in their acceptance of mates in such cases (Burley 1977; Andersson 1994; Amundsen 2000; Kokko & Johnstone 2002).

Mate quality

When a mate is chosen because it is of high "quality", this implies that it possesses attributes that should either (1) increase the survival or fecundity of the selecting individual (i.e. by providing direct benefits) or (2) increase the viability or attractiveness of progeny (i.e. by providing indirect genetic benefits). Mating preferences may thus be (1) directly selected (i.e. mates offer direct benefits, such as superior resources or parental care, that increase the survival or fecundity of the females that prefer them) (Heywood 1989; Hoelzer 1989; Kirkpatrick & Ryan 1991), or (2) indirectly selected (i.e. the genetic

benefits of choosing mates with particular attributes increase offspring fitness, either through increased viability or increased attractiveness; offspring inherit genes for both the preference(s) and the trait(s) associated with fitness) (Fisher 1930; Kirkpatrick & Ryan 1991; Eshel *et al.* 2000; Kokko *et al.* 2002). "Sexy sons" (or "sexy scions" in the case of species with mutual mate choice) are the inevitable result of a genetic correlation between a trait and a preference for this trait (Kirkpatrick & Ryan 1991; Kokko *et al.* 2003). It has also been suggested that preferences may evolve exaptively when a sensory system is adapted for non-mating contexts, but mates possess a trait that "exploits" this pre-existing sensory bias (Endler & Basolo 1998). Another recent model of sexual selection considers cases in which male signals coevolve with female resistance to these signals (the antagonistic chase-away model: Holland & Rice 1998; reviewed in Chapman 2003). The model suggests that female resistance evolves as an increased receiver threshold designed to limit the number of matings undertaken, which means that male signals must evolve to increase in intensity. In resource-based mating systems in which animals show biparental care, however, it is expected that direct benefits will generally dictate mate choices (Kirkpatrick & Ryan 1991).

Mate choice may be based on multiple cues in many species (Candolin 2003). In other words, an individual's final mate choice can be determined by several preference functions. Mate choice cues include phenotypic traits or resources defended by a potential mate that indicate its reproductive value (Candolin 2003). Cues that have been modified specifically for communicating mate quality to the opposite sex are commonly labelled as signals (Candolin 2003). When multiple cues provide information about mate quality, they may reflect different aspects of quality or may include back-up cues that reinforce other cues. A recent model concluded that the evolution of preferences for multiple indicators of quality is most likely when these cues provide independent information about different aspects of quality (van Doorn & Weissing 2004).

Preferences for ornamental signals such as bright colours, behavioural displays and exaggerated tails are common. These ornaments may reflect genetic quality (which would increase offspring viability (Zahavi 1975; Hamilton & Zuk 1982) or "sexiness" (Fisher 1930)) (Kirkpatrick & Ryan 1991) or they may act as "condition-indicators" (Price *et al.* 1993) or honest indicators of parental care capability (Motro 1982; Hoelzer

1989). Among species that exhibit mutual mate choice, both sexes may be ornamented (Amundsen 2000). Traditionally female ornamentation was thought to result from a genetic correlation with sexually selected male traits (Lande 1980). The fact that males prefer more ornamented females in some cases (Jones & Hunter 1993; Amundsen *et al.* 1997; Amundsen 2000) suggests, however, that selection can operate on female traits. There is, nevertheless, mixed evidence that female ornaments reliably signal quality (Amundsen 2000).

Variation in mating preferences within the same population often exists (Widemo & Sæther 1999; Forstmeier & Birkhead 2004). Choosiness may vary, depending on social, environmental and physiological constraints (Widemo & Sæther 1999). For example, trade-offs between the costs and benefits of choice may differ between individuals because of age, experience or body condition (i.e. condition-dependent choice: Jennions & Petrie 1997). High quality choosers are also expected to be choosier than lower quality individuals (i.e. assortative mating for quality: Burley 1977; Burley 1983). The other facet of mating preferences, preference functions, may also vary between individuals (Wagner 1998). For example, there is evidence that animals sometimes search for genetic (Mays Jr & Hill 2004) or personality compatibility (Tregenza & Wedell 2000). It has been argued that personality matching may be especially important among animals that form long term social partnerships (Forstmeier & Birkhead 2004). In addition, previous experience, including early-life experiences such as imprinting periods, may help to create or modify preference functions (Widemo & Sæther 1999).

Lifelong mate choice in socially monogamous species

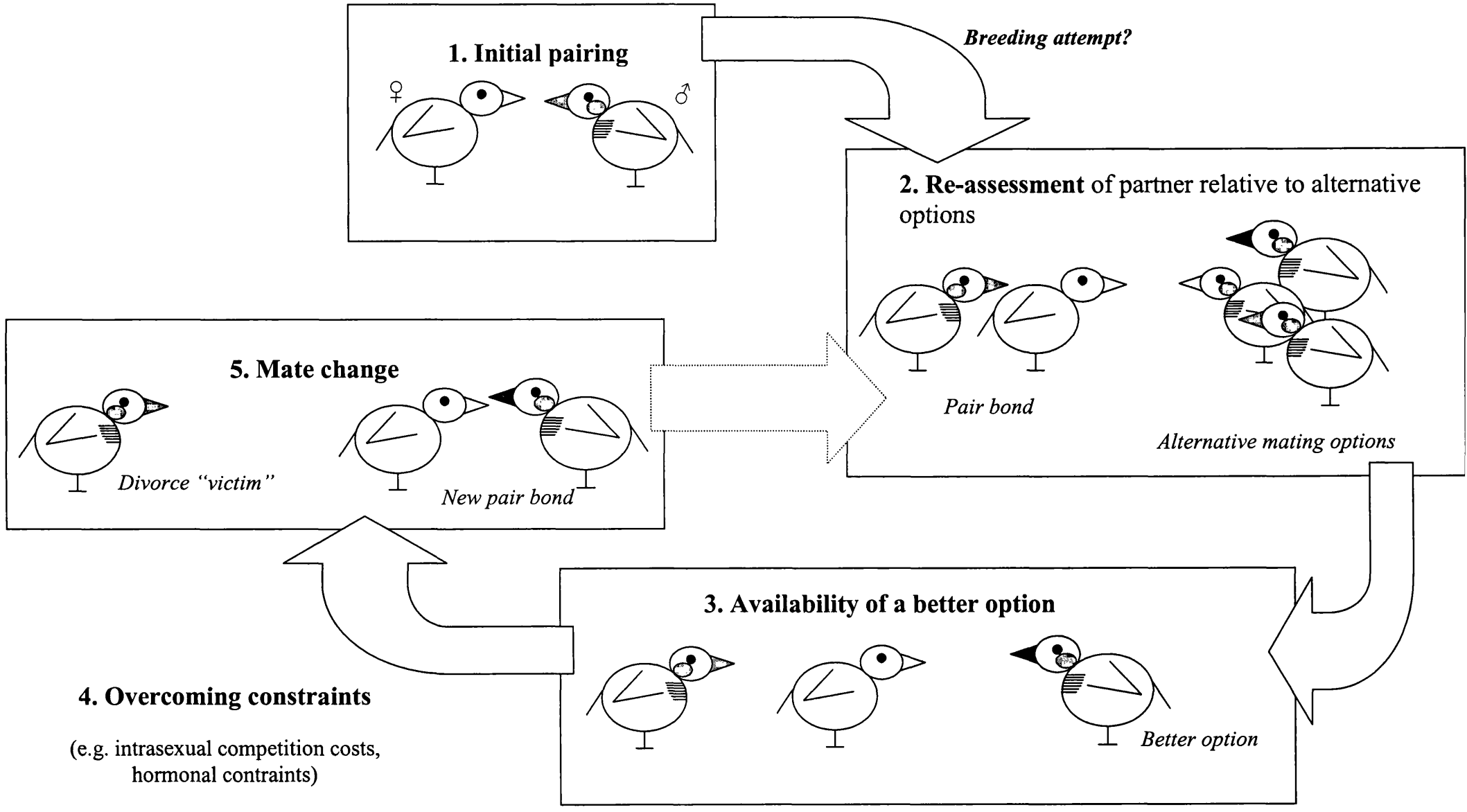
Mates often associate for longer than the duration of a single copulation; some form socially monogamous partnerships during one or more breeding events, which often involves co-operation between a male and a female to rear offspring (Wickler & Seibt 1983; Gowaty 1996). The evolution of social monogamy is traditionally considered to originate from the requirement for male parental care. Species with biparental care, however, show notable variation in the extent to which females can compensate for a lack

of male care (Gowaty 1996). When male care is found not to be essential, social monogamy is usually explained by evoking constraints such as female-female aggression or the inability of males to monopolise environmental resources and thereby females (Gowaty 1996). Social monogamy is very seldom observed in reptiles (Burley & Johnson 2002) or amphibians (although see Gillette *et al.* 2000), is rare among mammals (it occurs in less than 5% of species: Clutton-Brock 1989), fish (it has been observed in 18 families of marine fish, Whiteman & Côté 2004) and invertebrates (Black 1996), but rife among birds (occurring in approximately 90% of avian species: Black 1996).

The duration of social attachments (which are generally referred to as pair bonds) varies between species. Perennial monogamy involves long-term pair bonds that persist for more than a single breeding season and this type of social monogamy is present in 21% of bird families (Black 1996). Birds that maintain long-term pair bonds can obtain fitness benefits when mate familiarity improves breeding performance (Ens *et al.* 1996). Mate choice, however, can be regarded as a process that continues throughout life (Black 1996). As such, pair members can decide to change mates, either by maintaining a social pair bond and engaging in extra-pair copulations (EPCs) (reviewed in Westneat *et al.* 1990) or by severing the bond and divorcing their established partner (reviewed in Choudhury 1995). Divorce and EPC can thus be considered extensions of the initial mate choice process, in which individuals re-assess the quality of their partner relative to other available mating opportunities (Choudhury 1995; Black 1996) (figure 1.1). The viability of an individual's offspring may be increased by the indirect or direct benefits gained from breeding with a mate of higher quality than their established partner. However, mate change may involve costs such as the risk of losing territory or missing a breeding attempt, initial breeding inefficiency with a new partner, search costs and, among males, the requirement to trade-off mate guarding with EPCs (Birkhead & Møller 1992; Choudhury 1995). The balance between the benefits and costs of both fidelity and mate change will determine the mating decisions of birds (Birkhead & Møller 1992; Ens *et al.* 1996; McNamara & Forslund 1996).

Extra-pair paternity among bird populations ranges from 0 to 60% of offspring (Birkhead & Møller 1992). Divorce rates also vary considerably, ranging from 0% in species such as Bewick's Swan *Cygnus bewickii*, through to 100% in the House Martin,

Figure 1.1 A schematic representation of the component steps involved in adaptive divorce, illustrated with zebra finches.



Delichon urbica (Ens *et al.* 1996). Much of this variation can be explained by interspecific differences in life history and ecology. For example, life history and ecological constraints can result in non-adaptive divorce. This may involve the intrusion of a third individual, accidental loss or may occur as a side effect of differential arrival to breeding grounds (reviewed in Choudhury 1995). On other occasions, divorce can represent an adaptive reproductive strategy employed by an individual in order to improve its current breeding situation (Choudhury 1995). The idea of divorce ensuing from individual strategies has become particularly prominent in recent years (Ens *et al.* 1996; Cèzilly *et al.* 2000; Dhondt 2002). For example, pair members may initiate divorce if a better option is available to them (the better option hypothesis: Ens *et al.* 1993). Pair members may also consider their prior reproductive performance with an established partner relative to other pairs when re-assessing mate quality. There is a substantial body of correlative evidence suggesting that reproductive failure encourages divorce (see Dubois & Cézilly 2002 for a meta-analysis). However, there have been few experiment tests of the potential causes of divorce.

Early development and adult attractiveness

The environment experienced during ontogeny can have pronounced effects on the adult phenotypes of animals (Lindström 1999). Detrimental conditions during early development (i.e. the period from conception to adulthood: Henry & Ulijaszek 1996) can reduce both growth (Henry & Ulijaszek 1996) and sexual attractiveness (Gustafsson *et al.* 1995; Møller & Thornhill 1998). A well-studied example of an adverse early environment is one in which nutritional quality is low. The quality of nutrition received early in life can alter adult attractiveness (Ohlsson *et al.* 2002) and also traits relating to longevity (Desai & Hales 1997; Aihie Sayer *et al.* 2001; Aihie Sayer & Cooper 2002), both of which are components of lifetime reproductive success. Although some animals reach normal adult size and appearance after a developmental nutritional deficit (Arendt 1997), an ostensibly normal phenotype can conceal long-term problems accruing from this deficit (Lucas *et al.* 1996). Furthermore, it is now apparent that compensatory growth

once a nutritional restriction has abated can itself carry long-term costs (Metcalfe & Monaghan 2001, 2003).

The effect of early life conditions on avian song development has attracted much attention in the last few years (Nowicki *et al.* 1998). Male song in many species is used by females as an indicator of male quality (reviewed in Searcy & Andersson 1986; Catchpole 1987). More recently, this idea has been expanded upon with the formation of the nutritional stress hypothesis (Nowicki *et al.* 1998). This hypothesis is rooted in the strong influence exerted by developmental environments on adult attractiveness. For example, females may utilise learnt features of male song, such as repertoire size, as honest indicators of mate quality. The areas of the brain that control song learning, principally the high vocal centre (HVC), develop when birds are growing rapidly and are particularly sensitive to nutritional deficit (Alvarez-Buylla *et al.* 1992). Variation in adult song performance should thus reflect variation in the development of these song-learning centres, which in turn is indicative of the variation in responses of individuals to nutritional stress. Song repertoire may consequently indicate several aspects of male quality, such as a male's overall phenotypic condition as a result of its developmental conditions (Searcy *et al.* 2004). Adult phenotypic condition, in turn, is likely to affect parenting ability (Buchanan & Catchpole 2000). In addition song may indicate underlying heritable aspects of parental care (Nowicki *et al.* 1998) and genotypic differences in responses to nutritional stress (Williams 1966).

Zebra finches as a model system

I chose to use domesticated zebra finches, Taeniopygia guttata, as a model system for the experiments detailed in this thesis. Zebra finches are granivorous passerines that are found throughout continental Australia and the Lesser Sundas archipelago. They are a socially monogamous, but sexually dimorphic, species in which both sexes contribute substantially to parental care (i.e. wild birds show no sex differences in incubation behaviour or nestling feeding rates, Zann 1996) and they exhibit mutual mate choice. As several of my experiments were breeding experiments, these birds were a suitable study species because they reproduce rapidly and potentially all-year-round in captivity (Zann

1996). Zebra finches have been widely used as a model species for the study of sexual selection by female choice (Zann 1996), avian song learning (Slater *et al.* 1988) and the downstream effects of early nutrition (for example, de Kogel 1997; Birkhead *et al.* 1999; Blount *et al.* 2003a). Thus, for several of the principal topics that this thesis encompasses, there are substantial bodies of prior work on zebra finches.

Both sexes are known to discriminate between potential partners (Wynn & Price 1993; Clayton 1990). Indeed, Wynn & Price (1993) found "no obvious differences between the sexes in the strength of their choice". Such mutual mate choice was an important consideration for my experiments on pair bond stability (chapters 3 and 4), as this allowed for a comparison of male and female responses within pair bonds. Zebra finches have continuous, year-round partnerships and are traditionally presumed to have strong pair bonds (Butterfield 1970; Zann 1996). No observations of divorce have been made in any of the zebra finch colonial populations in Australia (Zann 1996). However, as Clayton (1990) indicates, this has not been investigated with thoroughness sufficient to exclude the possibility of divorce in the wild. This nevertheless raises the question of whether it is a suitable species with which to study pair bond stability? Zebra finches have a high mortality rate (Zann 1996). This is expected to increase divorce rates within populations because the vacancies created represent opportunities to improve mates at a low cost (Ens *et al.* 1993). In addition, as a colonial species, zebra finches would experience relatively low search costs, when locating a new mate, which is expected to increase divorce rates (Choudhury 1995). However, as a non-migratory species exhibiting continuous partnerships, one may expect, conversely, low divorce rates among zebra finches (Ens *et al.* 1996). Divorce, however, has been observed in captivity "for unknown reasons", as Nancy Burley describes it (Morris 1954; Burley 1986). Moreover, rates of extra-pair copulation (EPC) have been studied fairly extensively. Not all pairs are sexually monogamous; 8% of broods in the wild (Birkhead *et al.* 1990) and 11% of captive broods (Birkhead *et al.* 1989) display extra-pair paternity. As outlined in the relevant chapters, I measured pair bond stability, but did not make assumptions about the result of reduced stability (i.e. either EPCs or divorce). The use of a model system with known divorce rates and higher EPC rates may have been more suitable for the pair bond stability experiments. The selection of the zebra finch as my model system was a trade-

off between a comparatively low level of knowledge of its pair bond stability and the aforementioned benefits that it provides.

Mating preferences in zebra finches *Taeniopygia guttata*

The mating preferences of zebra finches are generally measured in preference-chambers by recording the amount of time spent affiliating with each of the available mating options (for example, Burley *et al.* 1982; ten Cate & Mug 1984; Burley & Coopersmith 1987; Wynn & Price 1993; Vos 1995; de Kogel & Prijs 1996; Blount *et al.* 2003a; Blount *et al.* 2003b). The proportion of time spent with a potential mate appears to be a reliable measure of mating preference as it reflects eventual pair formation for both sexes (Clayton 1990), correlates with the rate of female tail quivering (i.e. courtship behaviour) (ten Cate & Mug 1984; de Kogel & Prijs 1996) and predicts EPC preferences (Houtman 1992).

Studies of female mate choice in zebra finches have focussed on three preference functions – male bill colour, song rate and song syntax. Males have red bills that are carotenoid-based indicators of condition (Blount *et al.* 2003b). Song rate also appears to be a condition-dependent trait (Birkhead *et al.* 1998). Price (1996) found that additive genetic variation explains approximately 50-55% of phenotypic variation in bill colour, suggesting that bill colour is heritable (also see Price & Burley 1993). Contrary to this, however, Houtman (1990) suggests that song rate, but not bill colour, is heritable. Although adult bill colour may have a heritable component, it also seems to be influenced by both early (de Kogel & Prijs 1996) and current environment (Blount *et al.* 2003b). Several studies have found that females prefer males with comparatively red bills (Burley & Coopersmith 1987; Houtman 1990; de Kogel & Prijs 1996; Blount *et al.* 2003b) and high song rates (ten Cate & Mug 1984; Houtman 1992; Collins *et al.* 1994; de Kogel & Prijs 1996; Balzer & Williams 1998; Forstmeier & Birkhead 2004). These two traits are often correlated (Houtman 1990; de Kogel & Prijs 1996), but when both have been measured, generally song rate is a better predictor of female preference than bill colour (Houtman 1990; Collins *et al.* 1994; Forstmeier & Birkhead 2004; but see de Kogel & Prijs 1996). This suggests that either there is a hierarchy of preference criteria, with

display rate prioritised above bill colour (Collins & ten Cate 1996), or that females need to learn the association between bill colour and display rate (Collins & ten Cate 1996). Females also prefer males that produce relatively complex songs (i.e. songs containing phrases with a large number of elements, which are sometimes called syllables) (Clayton & Pröve 1989). Song complexity may indicate male quality to females as it provides an honest indicator of developmental stress (Nowicki *et al.* 1998; Spencer *et al.* 2003). The importance of song complexity as a mate choice cue in relation to bill colour and song rate is unknown. In summary, female zebra finches rely on multiple mate choice cues (Candolin 2003) that may reflect slightly different aspects of male quality.

Fewer studies have examined the preferences of male zebra finches, but males have generally been found to prefer females in good nutritional condition (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001). Egg production and incubation are state-dependent in this species (Monaghan *et al.* 1996; Selman & Houston 1996; Gorman & Nager 2003). Females also have carotenoid-based bill coloration, although their bills are generally less red than those of males. Among females, bill colour and body condition generally are not associated (de Kogel & Prijs 1996), which suggests bill colour is an unreliable signal of female fecundity (Burley & Coopersmith 1987). Males are believed to prefer females with bill colours in the middle of the phenotypic range (Burley & Coopersmith 1987). However, there has been little investigation into how both male and female mating preferences vary between individual zebra finches (Forstmeier & Birkhead 2004).

Early environment & life history traits in zebra finches

The zebra finch has been used on a regular basis to study the effects of early nutritional environments on a range of traits. For example, the effects of developmental nutrition on adult appearance have been much studied in this species, but with variable results. Neonatal nutritional conditions have been found to have permanent effects on adult mass and body size (Boag 1987; de Kogel 1997), on mass but not body size (Birkhead *et al.* 1999) and on neither mass or size (Blount *et al.* 2003a). To further complicate matters, the nature of experimental manipulations (brood size manipulations: de Kogel 1997;

manipulations of diet quality: Boag 1987; Birkhead *et al.* 1999; Blount *et al.* 2003a; manipulations of diet quantity: Spencer *et al.* 2003) and the duration of the treatment periods vary considerably between experiments. The extent to which permanent effects on adult appearance are produced depends on the severity of the early nutritional deficit. For example, Boag (1987) manipulated diet quality for approximately 100 days post-hatching and found considerable differences in adult appearance. Blount *et al.* (2003a) manipulated diet quality for 15 days post-hatching and found no differences in adult appearance. It appears that zebra finches are able to compensate for a relatively moderate nutritional deficit during development after the nutritional restriction has been lifted.

Despite nutritionally-restricted zebra finches in many cases exhibiting growth compensation, relatively poor neonatal nutrition in this species has been shown to produce adverse effects on a range of life-history traits. For example, early nutritional deficit reduces clutch size among females (Haywood & Perrins 1992), lowers adult lipophilic antioxidant concentrations (Blount *et al.* 2003a) and increases mortality among adult males (Birkhead *et al.* 1999). Periods of nutritional stress that overlap with the development of the HVC song control nucleus limit the size of the HVC (Buchanan *et al.* 2004) and reduce the song repertoire of adult males (Spencer *et al.* 2003).

Thesis content

The first half of this thesis examines aspects of zebra finch mating preference that have been relatively neglected in species with biparental care. ***Chapter 2*** reports the results of an experiment that investigated the influence of female dominance status on male mate preference. ***Chapters 3 and 4*** examine factors that may affect mating preferences within pair-bonded birds. Specifically, ***chapter 3*** looks at how pair bond stability is affected by the nutritional condition of an established partner relative to an alternative mate. ***Chapter 4*** investigates the influence of reproductive performance on pair bond stability. In ***chapter 5*** I look at whether birds show a preference for either relatively young, inexperienced (i.e. naïve) mates or relatively old, experienced (i.e. competent) mates. Generally in these chapters I attempted to (1) examine both male and female preferences and (2) consider the characteristics of the birds whose preferences were being measured.

The latter half of the thesis examines the influence of developmental nutrition on various adult traits that may be implicated in mate choice. In ***chapter 6*** I look at the effects of neonatal diet quality on growth and adult appearance. ***Chapter 7*** presents the results of two experiments that investigated whether neonatal nutrition is associated with antioxidant capacity in adulthood. ***Chapter 8*** examines the links between neonatal nutrition, growth rates and (1) male song learning and (2) associative learning performance among both sexes. In the final chapter (***chapter 9***) I discuss why variation in zebra finch mating preferences may exist, both within and between the experiments presented in this thesis, and I compare my results with previous studies of zebra finch mating preferences.

Methodological notes

Mate preference tests

I measured mate preferences using dichotomous choice chambers. I conducted three tests simultaneously, in the same room, to ensure that the zebra finches remained in acoustic contact. Choosing birds were presented simultaneously with two stimuli (i.e. mating options) during tests and preferences were inferred from the proportion of time spent affiliating with each option. This test design is commonly used, principally because the tests are easy to conduct and the data that they generate are relatively easy to analyse (Wagner 1998). As I previously discussed, the results of such tests predict final pair formation (Clayton 1990) and EPC activity (Houtman 1992) in zebra finches. They also have the advantage of separating mate preference from the effects of intrasexual competition (Qvarnström & Forsgren 1998). Some studies assess preferences in natural conditions (Andersson 1994) but these cannot separate preferences and intrasexual competition.

I recognise, however, that my two-stimulus, simultaneous presentation test design does have limitations. For example, this approach did not permit quantitative measurements of individual preference functions. The shape of a preference function can be measured by presenting individuals with sequential, single stimuli that differ in the strength of the trait being examined (Wagner 1998). The methodology that I adopted only

allowed as assessment of directional preference. Inferring directional selection in a two-stimulus design, in which traits at the extremes of a range are presented, is dangerous because the chooser's response to intermediate trait values is unknown (Wagner 1998). In some experiments, this may not have been important (e.g. the measurement of pair bond stability: chapters 3 and 4). However, sometimes preference functions may not be directional. For instance, when comparing mating preferences for competent and naïve mates (chapter 5), ideally choosers would have been presented with a range of mating options that represented various ages/experience levels. Absolute preference function can be measured by presenting a series of single stimuli of different trait values. Alternatively, relative preference function can be measured, which involves a comparison of the difference in trait value between two stimuli and the difference in time spent with the two mating options (Wagner 1998). However, the availability of suitable birds often limited the scope for assessing individual preference function in my experiments.

Simultaneous stimulus presentation can also provide a false impression of the strength of preference (Wagner 1998). For example, a chooser might respond strongly to two potential mates when they are presented singly, but might show a marginally stronger response to one. When the options are simultaneously presented, the chooser may affiliate to a much greater extent with the preferred option, thereby giving a false amplification of the preference strength. However, the presentation of sequential stimuli has attendant problems itself. Individual variation in response to single stimuli may be low and responses to isolated stimuli may not always predict mate choice accurately (Wagner 1998). Importantly, the prior mating options to which an individual has been exposed may affect their responsiveness during sequential stimulus presentations. For example, female zebra finches adjust their preferences for display rate in relation to previous experience (Collins 1995). In some cases I was able to measure variable relative preference functions (Wagner 1998), albeit at a population level rather than an individual level. This involved comparing the difference in affiliation time with two simultaneously presented mating options and the difference in trait value between the options.

As I discuss in chapter 9, many studies analyse population-level preferences and do not consider between-individual variation in preferences (Widemo & Sæther 1999). The consideration of preference variation is certainly important in zebra finches, given

the low between-female agreement regarding male attractiveness (Forstmeier & Birkhead 2004). Ignoring preference variation can result in a false conclusion that a trait is unimportant in mate selection, by masking preferences held by subsets of a population. For example, males of a poeciliid fish, *Brachyrhaphis rhabdophora*, appeared to have no preference for larger females when data was analysed at a population level. But male preference was dependent on their size: larger males preferred large females (Basolo 2004). I used a similar approach to Basolo (2004) by examining how mate preferences varied with chooser characteristics within a population (for example, I examined the influence of chooser age and activity level). Ideally, choosers would have been tested with the same test dyads more than once because, amongst females at least, the consistency of zebra finch mating preferences is low ($r = 0.29$, Forstmeier & Birkhead 2004). However, I decided instead to employ relatively lengthy test durations (90-180 minutes), using the rationale that longer test periods should improve the accuracy of mate assessment (Sullivan 1994). It is worth noting that Forstmeier & Birkhead (2004) used unmanipulated stimulus males when assessing mate preference repeatability. As a result, trait variation between males was liable to be low, making it more difficult for females to discriminate between potential mates. This may, in part, explain the low female consistency of preference in that experiment.

Two further issues regarding preference test methodology are worthy of discussion. First, mate preference test duration varies considerably in experiments across all taxa, as well as specifically in zebra finch experiments (see chapter 9 for a more detailed discussion). The provision of longer assessment periods may lead to a more accurate reflection of preferences (Sullivan 1994). However, experimental designs will be a compromise between the accuracy of preference measurement and an attempt to reduce time constraints. It would be useful to decide objectively on an optimal test length, for zebra finches, but also for any species being studied. This could be achieved by analysing how the rate of switching between mating options changes over time. This may allow the calculation of a test duration that captures a chooser's definitive preference, but also optimises the number of tests that can be conducted within a set time frame. Second, it remains unclear what affiliation time during a preference test actually indicates. Using the definitions of Jennions & Petrie (1997) and Widemo & Sæther (1999), a simultaneous

stimulus test should provide an indication of mating preference (i.e. a chooser's response to the two stimuli). This is a result of the order in which they rank the two available mating options, the strategy that they use to sample the options and their choosiness (i.e. their investment in mate choice). However, this may not always reflect final mate choices (and therefore the mating pattern) within a population, which will additionally be influenced by social constraints and competition over mates (Widemo & Sæther 1999). Ideally, experiments could test mate choices using several steps: first, an assessment of mating preference when mating options are isolated; second, an assessment of preference when the chooser can observe the options interacting; third, an assessment of pair formation when the chooser is allowed free access to all mating options; fourth, an assessment of mating patterns when choosers face intrasexual competition. However, this would extend the duration of experiments considerably.

Body condition index

I use a body condition index in conjunction with body mass in my analyses of mate preferences. This index is calculated as the standardised residual of body mass on structural size measurements (usually tarsus length and wing length) (Lindström & Piersma 1993). The intention of this index is to provide an estimate of stored protein and fat reserves, independent of structural size. This index has been used previously in studies of captive zebra finches (de Kogel & Prijs 1996). However, its use has criticised, particularly when employed in captive studies, as it assumes that all non-structural mass is useful. A proportion of non-structural mass in caged animals could constitute excess fat. Furthermore, the use of this body condition index assumes there are no sex differences, and little between-individual variation, in body shape. In fact, within one population of domesticated zebra finches, males had smaller skeletons than females, but possessed larger bills (Boag 1987). Consequently, I employ this condition index with caution. Nevertheless, in all my experiments, this measurement of body condition was highly correlated with body mass and produced qualitatively the same results during data analysis.

Statistical model selection

The two competing approaches to the analysis and interpretation of biological data are null-hypothesis testing and information-theoretic model comparison (Johnson & Omland 2004; Stephens *et al.* 2005). Throughout this thesis, I rely on the null-hypothesis testing approach. Model comparison is often appropriate when competing hypotheses are generated from the collection of a wide range of variables in observational studies (Johnson & Omland 2004). However, I considered that my experimental data was more suited to null-hypothesis testing. My data generally consisted of a fixed factor, which was the explanatory variable that I was testing (e.g. dominance status; breeding success), and a set of covariates that I considered may additionally influence the independent variable and thus required statistical control. I adopted Crawley's rationale of model simplification to produce a parsimonious model (Crawley 2002). I used backward elimination of least significant terms in a saturated model, to produce a simplified, minimum adequate model containing significant variables.

Chapter 2

Female dominance status, body mass and male mate preference

ABSTRACT

In species exhibiting biparental care, both dominance status and reproductive potential may influence mating preferences. In some cases, however, dominant individuals are poor parents and may not be preferred as mates. The interaction between intrasexual competition and mate choice has received little attention in socially monogamous species, particularly in terms of male mate choice. Here I show that male zebra finches, *Taeniopygia guttata*, prefer females in good body condition in preference tests, but they do not discriminate between dominant and subordinate females. Dominant females did not lose body mass in relation to subordinate birds, which suggests that the establishment and maintenance of dominance status is not costly for females in terms of nutritional condition. The ability to discriminate between females based on their capacity to maintain body mass appeared to improve with both male age and the effort invested by males in mate assessment.

INTRODUCTION

Animals often discriminate between mates when individuals within their pool of potential partners vary in quality (Parker 1983). The degree to which each sex is choosy (i.e. the degree of effort they invest in mate assessment: Jennions & Petrie 1997) will depend both on the variance in mate quality among the opposite sex (Parker 1983) and on the costs borne during a single breeding event (Kokko & Monaghan 2001). Thus, when males and females have comparable costs of breeding (i.e. when both rear offspring and contribute similar levels of parental investment (PI: *sensu* Trivers 1972)) both are likely to be selective to some extent over the quality of mate that they find acceptable (Burley 1977; Andersson 1994). This is particularly the case amongst bird species, over 90 % of which exhibit social monogamy (Black 1996). Most studies of socially monogamous species have, nevertheless, focussed on female choice and there have been comparatively few studies of male mate choice.

Females that provide males with direct benefits, in the form of high fecundity and good parental care, are likely to provide the highest fitness returns and they should be favoured as mates. The potential direct benefits offered by a female may be indicated by physical traits such as body condition and size. In ectothermic taxa, larger females are generally more fecund and are preferred by males over smaller females (for example, Gwynne 1981; Verrell 1982; Côte & Hunte 1989; Olsson 1993; Kraak & Bakker 1998). The relationships between body size, fecundity and body mass appear to be more complex in birds (Blanckenhorn 2000). Estimates of nutritional reserves (i.e. body condition) can be made by calculating residual body mass from body size and are associated with breeding performance in some avian species (for example Sæther *et al.* 1997; Blums *et al.* 2002; but see Winkler & Allen 1996). Body size itself may affect egg formation through nutrient storage capacity (Alisauskas & Ankney 1990) and offspring rearing through foraging efficiency (Barbraud *et al* 1999). Males have been shown to prefer large females (Amundsen *et al.* 1997) and fecund females (Monaghan *et al.* 1996; Jones *et al.* 2001). However, evidence for fecundity-related male preference remains sparse because relatively few studies have examined male mating preferences.

It appears that there is an equivocal relationship between reproductive value and dominance status (Qvarnström & Forsgren 1998). On the one hand, dominant

individuals may have priority of access to food resources and preferred habitats (Peters & Grubb Jr 1983; Ekman & Askenmo 1984; Hogstad 1988; Wiley 1991), be better able to maintain protein reserves (Carrascal *et al.* 1998) and fat reserves (Piper & Wiley 1990; but see Gosler 1996), have improved overwinter survival (Kikkawa 1980; Arcese & Smith 1985) and reduced predation risk (Hegner 1985). These fitness advantages may allow dominant individuals to provide direct benefits to mates, in the form of high quality resources and parental care. As a result, dominance and parental ability can be correlated (Bisazza *et al.* 1989; Qvarnström & Forsgren 1998).

On the other hand, dominant individuals are not always especially good parents (Forsgren 1997; Qvarnström 1997; Qvarnström & Forsgren 1998) and the relationship between dominance and condition appears to be variable. Dominance can be metabolically expensive (Hogstad 1987; Bryant & Newton 1994). This can impair the nutritional condition of dominant individuals (Hay *et al.* 2004), which is liable to adversely affect their ability to provide parental care. In some cases, the effort invested in achieving dominance may be traded off against parental investment and in species in which good parents are preferred as mates, dominant individuals are less likely to be preferred (Qvarnström & Forsgren 1998). Whether or not a trade-off exists, and the consequences this has for mate choice, may depend on life history traits and environmental factors that will differ between species and populations (Qvarnström & Forsgren 1998).

Few studies have addressed how dominance status affects the sexual attractiveness of either sex in species with biparental care. Johnson (1988) showed that the preferences of male pinyon jays, *Gymnorhinus cyanocephalus*, are based on a combination of female mass, dominance rank and size. These traits had an additive effect and mass appeared to be the most important single component. However, only 60 % (9 of 15) of the preferred females were dominant and the comparative importance of female-female competition and male preference are unclear in this experiment. Females were allowed to interact during male preference tests and in some cases intrasexual competition can override mate preferences (Qvarnström & Forsgren 1998).

Male and female zebra finches, *Taeniopygia guttata*, exhibit similar levels of PI (Zann 1996) and both sexes discriminate when pairing with social mates (Burley *et al.* 1982; Burley & Coopersmith 1987; Wynn & Price 1993). Indeed, the strength of mate choice seems to differ little between the sexes (Wynn & Price 1993). In zebra

finches, reproductive effort is state-dependent (Monaghan *et al.* 1996; Selman & Houston 1996; Gorman & Nager 2003) and male zebra finches have been shown to prefer females in good nutritional condition (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001). The effect of female dominance status on their attractiveness to males has not previously been investigated. In this study I examined the relationship between female dominance status and sexual attractiveness using standardised mate preference tests in which female-female competition was controlled. Males were presented with dyads of females in which dominance relationships had been established and were thereby allowed to choose between a dominant and a subordinate female.

METHODS

Experimental birds

Female zebra finches (n = 60) were paired into 30 dyads, each of which was housed in a 60cm x 45cm x 40cm cage. Females within each dyad had no previous contact with each other and had the same plumage colour (wild type grey or fawn). Females were aged between 10 and 28 months at the time of the preference tests. I grouped females (groups of 8-12 birds) of approximately the same age and formed dyads based on the rank order of body mass within these groups. Mass and age may influence both the dominance status and mate preferences of zebra finches (Schubert *et al.* 1989; Wynn & Price 1993; also see chapters 3, 4 and 5 of this thesis). Although asymmetries within dyads were reduced by matching females for these traits, and this may have reduced the chances of clear dominance relationships emerging, the chances of a male preference for dominance being confounded by mass or age were minimised. Indeed, dominant females were not heavier at the time of the preference tests (see Results section) or older (Wilcoxon signed ranks test: $Z = 0.49$, n = 24 dyads, $P = 0.627$) than subordinates. The female dyads were housed in a room (21.25 ± 0.08°C) without males, who were kept in single sex groups. All birds were fed on *ad libitum* mixed seed, containing yellow, red, pearl white, panicum, Japanese and Canadian canary millet (foreign finch mixture; Haith's, Cleethorpes, Lincolnshire, UK), cuttlefish, grit

and water. In addition, they received approximately 1.5g of conditioning food (which comprised Rearing and Conditioning supplement (J. E. Haith), moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had been diluted 1g/litre with water) twice per week and green vegetables once per week. The two females in each dyad were distinguished by the position of a red ring on either their right or left leg.

Assessment of female dominance status

Dominance status in birds is commonly inferred from contests over limited resources (for example, Piper & Wiley 1990; Belthoff & Gauthreaux Jr 1991; Dearborn & Wiley 1993; Bryant & Newton 1994; Cuthill *et al.* 1997). I recorded the use of limited resources as well as agonistic contests in situations of (1) restricted availability of a favoured perch and (2) restricted food availability. Dominance status within each dyad was assessed 8 to 10 days after the dyads were established.

I restricted the availability of perches in each cage by replacing the regular perches with two "single-seater" wooden perches. These perches were 50mm wide, which allowed only a single bird to perch at any time. One of these perches was positioned 120mm from the top of the cage near the back wall. The other perch was positioned 100mm from the cage floor and near the front of the cage. Birds prefer the high perch near the back of the cage because this is likely to provide a greater perception of safety (Swaddle 1996; Hawkins *et al.* 2001) and dominant birds were expected to spend more time on the high perch than subordinate birds. Initially the regular perches remained in the cages for two days and then on each subsequent day they were removed for approximately 5 hours each day in order to train the birds to use the single-seater perches. Each dyad was observed for three 30-minute periods during the afternoon on days 8, 9 and 10 post-pairing. I used a scan sampling protocol to record the position of the two dyad members (on the high perch, the low perch or the cage floor) every minute. During these observation periods, only the single-seater perches were present in each cage. Observations were made from behind a black screen, approximately one metre from the focal cages. I calculated the proportion of time that each dyad member spent on the high perch (for which I included only scan samples where one or other of the birds occupied the high perch). Neither female used the "single-seater" perches in 6 dyads. In the remaining 24 dyads, one or other of the

females used the high perch for 50.7 ± 7.3 % of the recording time. Time on the high perch was used as one measure of dominance.

I established which female won the majority of agonistic contests. A bird was deemed to have won an agonistic encounter when she supplanted her fellow dyad member from a perch or food bowl, or chased, pecked or beak-fenced the other dyad member, causing her to retreat (see Caryl 1975). I recorded contests during the three 30-minute observation periods in which perch behaviour was monitored. Agonistic interactions were recorded during the 20-second time slot immediately preceding each of the 30 scan samples. I recorded agonistic interactions around a limited food resource during a single 60-minute recording session on either day 8, 9 or 10. Food bowls were removed from dyads when the bird room lights came on in the morning. Following one hour of food deprivation, birds were given a food bowl that provided access to one bird at a time. The dyads were then filmed for one hour, and agonistic contests during this period were recorded. In total, contests were recorded over 150 minutes (i.e. 90 minutes with restricted perch access and 60 minutes with restricted food access). The bird that won the majority of contests was considered dominant. Contests were observed in 15 of the 30 dyads and dominance relationships in these dyads were obvious. In 13 of these 15 dyads, a single individual won every contest. In the remaining two dyads, one female won 9 of 10 contests and another female won 5 of 7 contests. When no contests were recorded, I considered the dyad member that spent most time on the favoured perch to be dominant (n = 9). This was justified by the correlation between contest wins and high-perch use (Spearman's rank correlation for the proportion of agonistic contests won by right-ringed females (arcsine square root transformed) and the proportion of time spent on the high perch by these females (arcsine square root transformed): $r_s = 0.600$, n = 15 dyads, $P = 0.018$). In 6 dyads, neither female used the high perch or engaged in agonistic contests.

Body measurements

I measured tarsus length from the nuchal notch to the end of the tarsus with the foot flexed at a right angle. Three measurements were taken for each bird, and these were then averaged. Body mass was measured to the nearest 0.1g at the time of pairing and again on the day of the mate preference tests. Both initial mass at pairing and mass at the time of the preference test were measured between 10:30 and 13:00. The

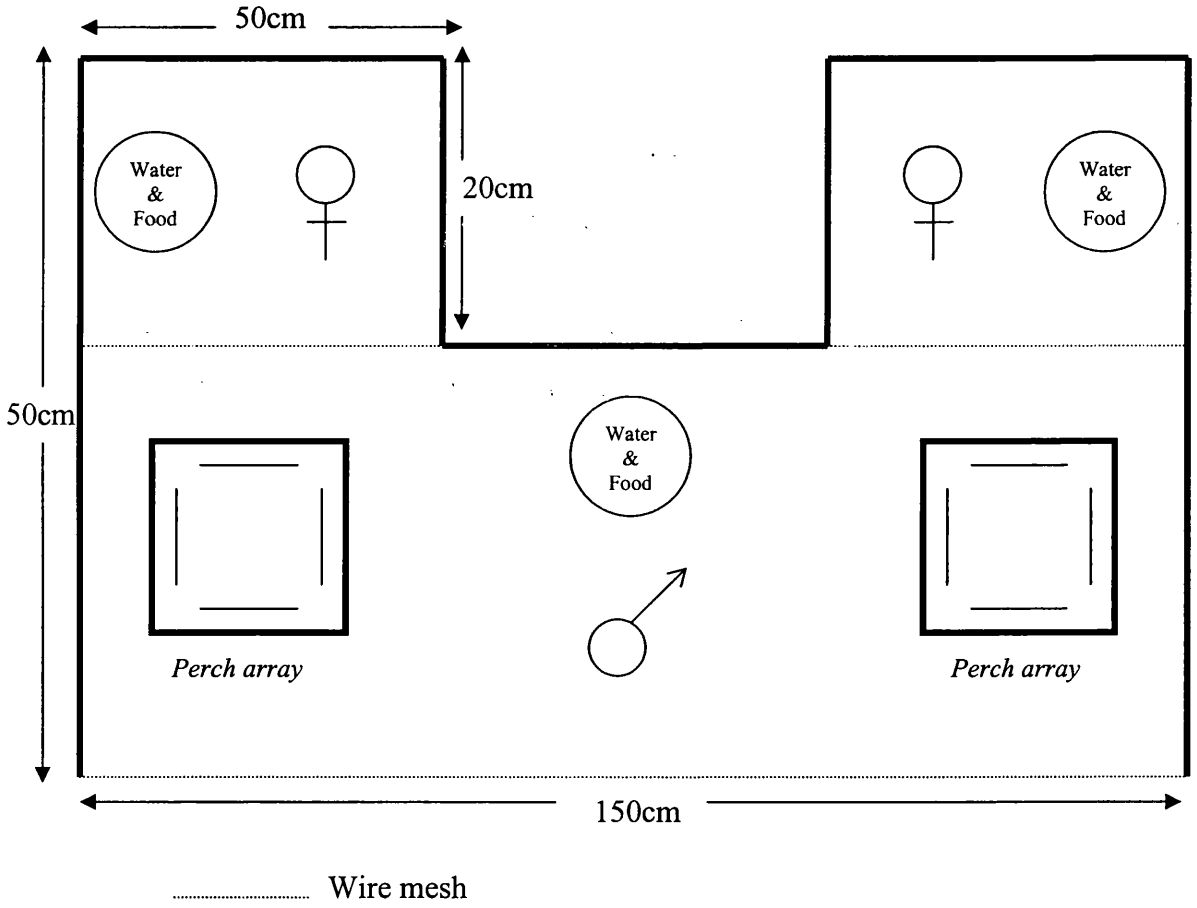
standardised residual of body mass regressed on tarsus length was used as an index of body condition (for example, Lindström & Piersma 1993). Three independent observers visually assessed bill colour within 7 days either side of the mate preference tests. The upper surface of the bill of each bird was compared to 13 colour chips that had values ranging from 1 (intense red) to 13 (pale orange). There was strong agreement between all three assessors ($r_s > 0.81$, n = 58 (no measurements for one dyad), $P < 0.001$), therefore mean values were used for subsequent analysis.

Mate preference tests

Each dyad was entered into a mate preference test, 11 days after females were initially paired. Choosing males, who had no prior contact with the experimental females, were given a choice between the two females from the same dyad. Males were between 10 and 31 months old at the time of testing. Tests were conducted in a two-way preference arena (figure 2.1) in which the two females could not see or interact with each other, which excluded the possibility of female-female interactions. Tests were conducted under full spectrum, artificial light (Bird Lamp; Arcadia, Croydon, UK). All three birds were allowed to acclimate in the test arena for one hour prior to the start of a three-hour recording period. The position of the choosing male was monitored by two microswitch perch arrays, one adjacent to each female, which were connected to a logger that recorded the time spent on each perch. A different male was used in each test, but males failed to use the perch arrays in 7 of the 30 tests and preference data were therefore only collected for 23 dyads. Dominance relationships were resolved for 17 of these 23 dyads. The proportion of the total test time spent on the two perch arrays was labelled the "active choice" time of a male. The proportion of active choice time spent with each of the females was deemed to be an index of mating preference. A female was judged to be preferred as a mate when the male spent more than 50 % of his active choice time with her. Time spent affiliating with an individual has been validated as a measure of sexual attraction in zebra finches, as it is correlated with female tail quivering (ten Cate & Mug 1984; de Kogel & Prijs 1996), predicts subsequent pair formation for both sexes (Clayton 1990) and predicts EPC preferences (Houtman 1992). In addition, the hop frequency (number of hops per minute) of males on each perch array provided a measure of courtship activity. Male hop rate was associated with the absolute amount of test time spent with each female

(r_s = 0.363, n = 43 (three females that were not visited by the male chooser were excluded), P = 0.017). This provided an additional endorsement of the use of affiliation time as a measure of mating preference.

Figure 2.1 Mate preference test arena. The lower half of the mesh separating the male chamber from the female chambers was covered. Therefore males were required to sit on the perch arrays in order to view females. Both female compartments contained two perches.



Statistical analysis

Analyses were conducted using SPSS (version 10.0, 1999). Non-parametric tests were used in cases where the data did not meet the assumptions of an equivalent parametric test. Percentage and proportion data were arcsine square root transformed prior to analysis when they were non-normally distributed.

I used a general linear model to determine whether the strength of male mating preference (i.e. the proportion of active choice time spent with the preferred female) was affected by any female traits. This analysis included 17 dyads (i.e. those for which both dominance data and male preference data were obtained). The dependent variable was the percentage of active choice time that choosing males spent with their preferred female. Dominance status (the preferred female was dominant or subordinate) was entered as a fixed factor. Within-dyad differences (preferred minus non-preferred female) in body mass at the time of the test (percentage mass difference, as a percentage of the lighter bird's body mass), age, mass change (percentage change in body mass between initial dyad formation and preference tests) and bill colour were entered as covariates. Males are expected to prefer intermediate female bill colour (Burley & Coopersmith 1987). I calculated median female bill index score (7.33, n = 58) and the deviation of each bird's bill index score from this median. Smaller deviations were expected to be more attractive to males. I ran the model again using the within-dyad difference (non-preferred minus preferred female) in deviation from the median bill colour as an independent variable, but this did not qualitatively alter the result of the model. Entering within-dyad differences in body condition or tarsus length instead of mass also did not qualitatively change the model results, and these results are not shown. I entered interactions between dominance status and other female traits into the initial model.

I used logistic regression analysis to test which female was preferred by the choosing male. The dependent variable was male preference (the dominant female within a dyad was either preferred or not preferred). Male chooser age, body mass and activity level (i.e. "active choice" time: the proportion of test time spent on the perch arrays) were entered as covariates. Within-dyad differences in body mass, percentage mass change over the course of the experiment, bill index score and age (all calculated as dominant female minus subordinate female) were entered as covariates. Entering within-dyad differences in body condition or tarsus length instead of mass did not

qualitatively change the model results, and these results are not shown. Similarly, the two calculations of relative bill colour (see above) did not produce different results. Stepwise backward deletion of variables with non-significant effects was used in both analyses, starting with interactions where $P > 0.100$. Means ± standard errors are presented in the text.

RESULTS

Dominant females (n = 24), on average, gained 3.54 ± 1.44 % of their original mass over the 11-day experimental period, whereas subordinate females (n = 24) maintained their original mass (mean loss of 1.06 ± 1.49 % of their initial mass) (Wilcoxon signed ranks test on the percentage changes in mass within dyads: $Z = 2.34$, n = 24, $P = 0.019$). Dominant females were not initially heavier than subordinate females upon pairing (paired t-test: $t_{23} = 0.22$, $P = 0.825$). Despite the slight changes in body mass, at the time of the mate preference tests dominant females were still not significantly heavier (paired t-test: $t_{23} = 1.67$, $P = 0.109$) or in better condition ($t_{22} = 1.15$, $P = 0.261$) than subordinates.

Males spent more time with the dominant female in 11 of 17 tests (two-tailed binomial test: $P = 0.332$) and hop rates did not differ in front of dominant and subordinate females (Wilcoxon signed ranks test: $Z = 0.93$, n = 17, $P = 0.352$). No variable that I tested predicted whether choosing males preferred the dominant female (table 2.1), although three marginally non-significant interaction terms are worthy of consideration. Both relatively old and relatively active male choosers generally preferred the dominant female within a dyad only when she was also the heavier dyad member, whereas relatively young and inactive males did not appear to discriminate between females in terms of body mass (figure 2.2). Male age was, however, not associated with male activity level ($r_s = 0.28$, n = 17, $P = 0.274$). Interestingly, relatively old males, but not younger males, tended to prefer the dominant female within a dyad only when she did not lose body condition in relation to her subordinate cage-mate (figure 2.3).

Males spent more time affiliating with the heavier female (paired t-test: $t_{22} = 3.09$, $P = 0.005$) and the female in better body condition ($t_{22} = 2.26$, $P = 0.034$) within

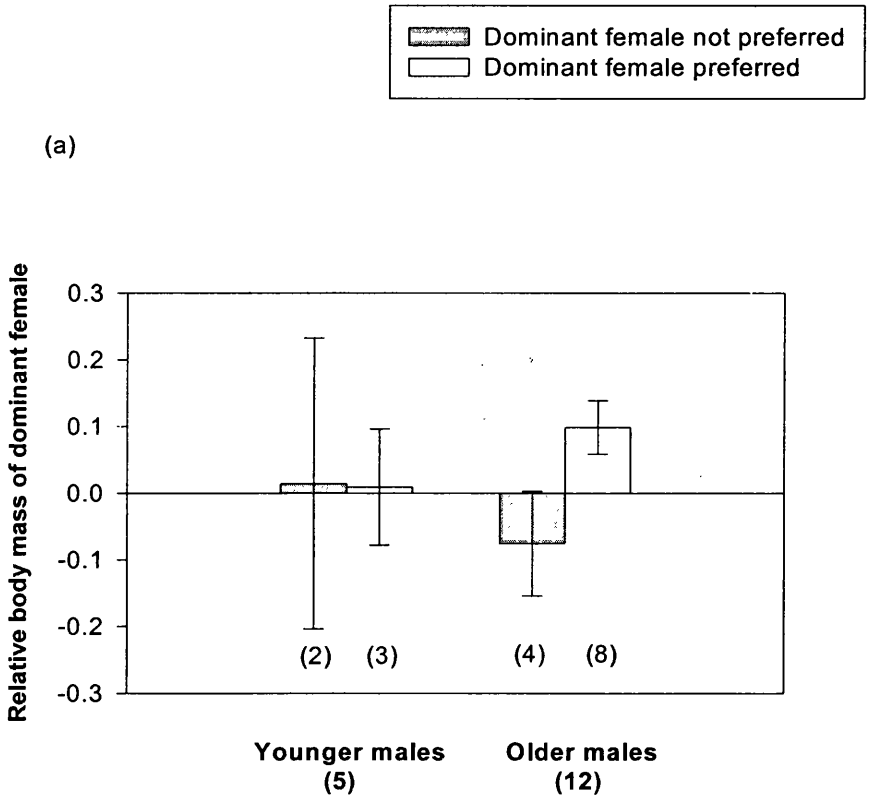
Table 2.1 Logistic regression analysis of the effects of male and female traits on the probability that the choosing male preferred the dominant female. The dependent variable was the male chooser's response to the dominant female (either the male preferred or did not prefer the dominant female). See Methods section for details of how independent variables were calculated. When relative female tarsus length or body condition were entered instead of relative mass, the results were not changed qualitatively. Other than those shown, for all interactions, $P > 0.300$. N = 17 preference tests.

Variable	χ^2	P
Relative female body mass	2.44	0.118
Relative female age	1.37	0.242
Relative % change in female body mass	1.58	0.209
Relative female bill index score	0.08	0.775
Male chooser age	0.35	0.552
Male chooser mass	0.98	0.323
Male chooser activity level	1.41	0.235
Male age x relative female mass interaction	3.58	0.059
Male age x relative % change in female mass interaction	2.73	0.099
Male activity level x relative female mass interaction	2.91	0.088

Table 2.2 Associations between traits of experimental females. Tests were Spearman's rank correlations (r_s values are given) and significance levels were corrected for multiple comparisons using a sequential Bonferroni test (the Dunn-Šidák method, Sokal & Rohlf 1995). N = 58 individuals. Body mass, condition and bill colour were recorded at the time of mate preference tests (see text). Asterisks denote significance at $P < 0.01$.

	Body mass	Body condition	Tarsus
Body condition	**0.85***		
Tarsus	**0.52***	0.04	
Bill colour	0.26	0.32	0.03

Figure 2.2 The effect of relative female mass on the attractiveness of dominant females in relation to (a) male age and (b) male activity level. Within-dyad differences in body mass were calculated as a proportion of the lighter dyad member's mass. Positive values indicate that the dominant female was heavier than the subordinate. Analyses used male age and male activity level as covariates. Here males are categorised for presentational purposes as relatively young (approximately 10 months) and relatively old (19 to 31 months). Similarly, males are categorised here as either relatively inactive (using the test perch arrays for < 40 minutes) or relatively active (> 40 minutes of perch use). Sample sizes are shown in brackets.



(b)

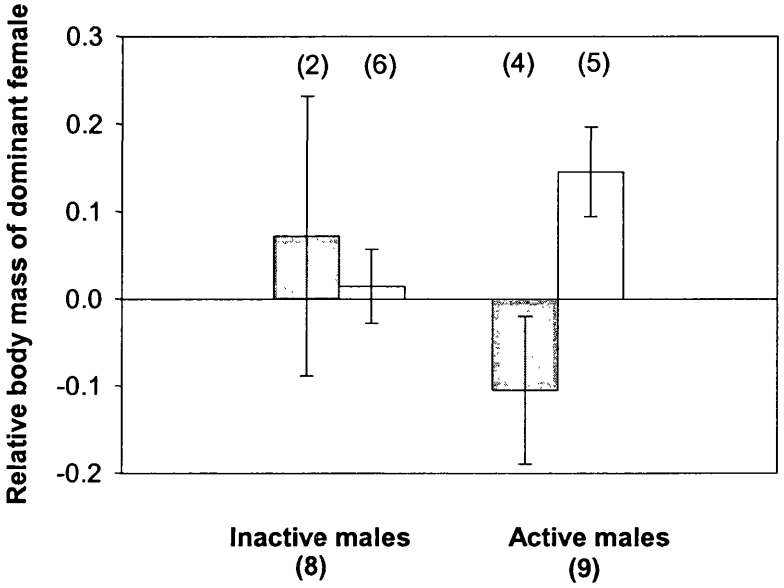
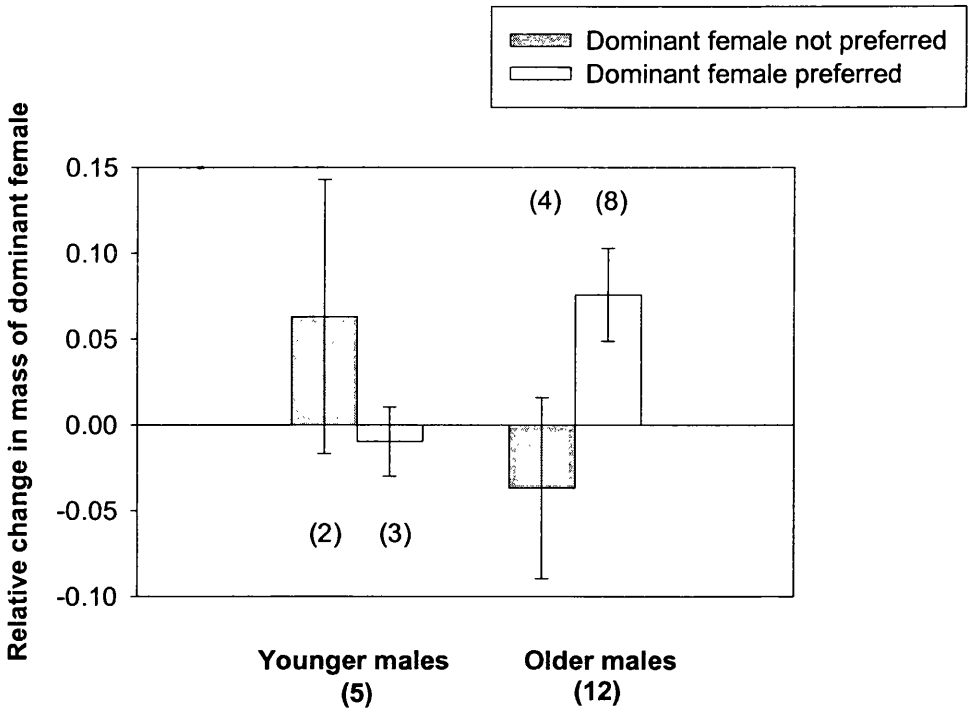


Table 2.3 Analysis of the effects of female traits on the time the choosing male spent with the preferred female. See Methods section for details of how independent variables were calculated. All interaction terms were non-significant ($P > 0.102$). When relative female tarsus length or body condition were entered instead of relative mass, the results were not changed qualitatively. N = 17 preference tests (including only preference tests for which dominance status was resolved within dyads).

Variable	*F*	*df*	*P*
Female dominance status	0.84	1, 13	0.376
Relative body mass	0.51	1, 15	0.485
Relative % change in body mass	< 0.01	1, 11	0.988
Relative bill index score	< 0.01	1, 12	0.977
Relative age	0.35	1, 14	0.563

Figure 2.3 The influence of the relative proportional change in body mass within dyads on the attractiveness of dominant females in relation to male age. Each female's proportional change in body mass between initial dyad formation and the preference tests was calculated; the within-dyad difference (dominant female minus subordinate female) is shown. Positive values indicate that the dominant female had a more favourable body mass change than the subordinate female over the duration of the experiment. Analyses used male age as a covariate. Here males are categorised for presentational purposes as relatively young (approximately 10 months) and relatively old (19 to 31 months). Sample sizes are shown in brackets.



each dyad. Preferred females were, on average, 8.07 ± 2.76 % (n = 23) heavier than non-preferred females. The majority of males spent over 50% of their time with the heavier female (16/22 birds, in one case both females in a dyad were of equal mass, two-tailed binomial test: $P = 0.052$) and with the female in better condition (18/23

birds, two-tailed binomial test: $P = 0.011$). This "population preference" for heavier females was a result of the test behaviour of relatively old (19 to 31 months) choosers (preferred female was 10.93 ± 2.66% (n = 17), one-sample t-test: $t_{16} = 4.12$, $P = 0.001$), but not relatively young (approximately 10 months) choosers (preferred female was 0.03 ± 6.87% (n = 6), one-sample t-test: $t_5 < 0.01$, $P = 0.997$). Hop rates, however, did not differ in front of heavier and lighter females ($Z = 0.19$, n = 22 (equal mass in one dyad), $P = 0.848$). Female bill colour was not associated with body condition once correlations between female traits had been corrected for multiple comparisons (table 2.2). Nor were females with redder bills preferred by males (12/23 dyads; two-tailed binomial test: $P > 0.999$). The proportion of active choice time spent by males with their preferred female was not associated with the extent to which females differed in any trait (table 2.3).

DISCUSSION

In this study, male zebra finches did not find dominant females more attractive than subordinates. This was the case in the absence of behavioural cues of relative dominance status, since the females were not observed interacting by the males. These results do not necessarily imply that males do not discriminate between dominant and subordinate females. However, if dominance status is important for male mate choice, males cannot deduce dominance status when females are alone. When males can observe female-female interactions, male preference may be modified or, alternatively, female dominance (intrasexual competition) may override male preference (Qvarnström & Forsgren 1998). Male pinyon jays, *Gymnorhinus cyanocephalus*, for example, prefer dominant females when they are able to see females interacting (Johnson 1988). Future mate preference studies should perhaps attempt to record both opposite-sex preferences and the outcomes of intrasexual competition over mates, as their results may not always be concordant.

It has been suggested that in some circumstances individuals may trade-off investment in dominance status against investment in parental care when both are costly (Qvarnström & Forsgren 1998). These trade-offs are likely to operate to some extent in females as well as males in species with biparental care. However, being

dominant was generally not costly for females in this study. There was actually a tendency for dominant females to improve their body condition over the 11-day experimental period relative to subordinate dyad members. The relationship between dominance, metabolic rate and condition varies between species and study conditions. In wild populations, the maintenance of dominance status can entail high metabolic rates (Hogstad 1987; Bryant & Newton 1994; but see Senar *et al.* 2000) and loss of nutritional condition (Hay *et al* 2004). Conversely, correlative field data often indicate that dominant individuals maintain nutritional reserves better than subordinates because of a feeding advantage (for example, Piper & Wiley 1990; Carrascal *et al.* 1998). It is improbable that dominant birds in this study held a feeding advantage because food was supplied *ad lib.* Both dyad members would have received adequate food, even if dominant birds gained priority of access to food resources. Differences in body mass regulation between dominants and subordinates may be best explained by differences in stress levels, metabolic rates or both. In most non-cooperatively breeding species that have been studied, subordinate individuals tend to have higher basal glucocorticoid levels (Creel 2001). This suggests that subordination can act as a stressor and subordinate individuals may be more vulnerable to stressful conditions (Senar *et al.* 2000). Subordinate white-throated sparrows, *Zonotrichia albicollis*, lose more weight overnight in captivity than dominant birds (Wagner & Gauthreaux Jr 1990) which suggests subordination may increase physiological stress or metabolic rate.

Cuthill *et al.* (1997) found that in zebra finches the effect of dominance status on mass depends on the time of day. Subordinate birds were more likely to be heavier at the beginning of the day, whereas dominant birds tended to be heavier at the end of the day. The mean difference in mass change between dominant and subordinate birds over the course of my experiment was small (4.60 ± 1.82 %), given that zebra finches can gain over 10% of their original body mass between dawn and dusk (Cuthill *et al.* 1997). I measured birds in the middle of the day, and perhaps by this time dominant birds had fed more than subordinates during the morning, if they indeed had priority of access to food. Had I measured body mass at dawn, I may have found no difference between dominants and subordinates. Cuthill *et al.* (1997) restricted food access throughout their experiment and interpreted the different daily patterns of body mass change of dominant and subordinate males as body mass regulation strategies. As dominants can monopolise food they can afford lower dawn reserves, whereas

subordinates should maintain relatively high dawn reserves because of a potentially unpredictable food supply. Food was unrestricted in this study, in contrast, except for the day on which agonistic interactions were recorded around a restricted food resource. Therefore these separate mass regulation strategies may not have been employed. Interestingly, in the study of Cuthill *et al.* (1997), dominant birds lost mass overnight in relation to subordinates, which is consistent with dominance requiring high energy expenditure. My observation that body condition tends to be maintained better by dominants may therefore have resulted from dominant females feeding heavily in the morning as a necessity to fulfil daily energy requirements. However, a reliable interpretation of these mass regulation results would require body mass to have been recorded in more detail, and possibly for patterns of daily feeding behaviour to be observed.

I found that males prefer heavier females and females in better condition (i.e. females that are heavy for their size) irrespective of the female's dominance status. These traits are indicative of fecundity. This result supports previous studies that have found a male preference for protein-supplemented females (Monaghan *et al.* 1996; Jones *et al.* 2001) and heavier females (Wynn & Price 1993). Males should gain direct benefits by selecting fecund females as mates and fecundity is likely to vary between individuals (Amundsen 2000). Zebra finches, however, to my knowledge remain the only avian species in which this preference has been shown. It is unclear how males assess female condition. Perhaps males are able to visually assess body condition, or condition may affect female courtship behaviour, vocalisations or posture. The preference for females in good condition is, however, not a result of direct interactions between the two females since it is maintained when no female-female interactions are possible (Jones *et al.* 2001), as was also the case in this study. The results of this study suggest, in addition, that the use of female body mass as a preference cue increases with both male age and activity level. The accuracy of mate-quality assessment may improve with both previous experience (Widemo & Sæther 1999) and the amount of time invested in assessment (i.e. "choosiness" *sensu* Jennions & Petrie 1997) (Sullivan 1994). Furthermore, it has been proposed that only the fittest individuals can cope with the costs of maintaining dominance without suffering reduced condition (Hay *et al.* 2004). My results suggest that old, experienced males may prefer dominant females that maintain their body condition, but that they tend to avoid dominant individuals that lose condition. However, this interpretation needs to

be treated with caution, given the marginal significance of the interaction (male age by relative female change in mass: $P = 0.099$).

Bill colour is a cue that males could potentially use in mate selection. There is mounting evidence that carotenoid-based male ornaments can signal male condition to females (for example, Peters *et al.* 2004). The red bill of male zebra finches appears to be a good example of such an ornament (Blount *et al.* 2003). However, very few studies have investigated the condition-dependence of female ornaments (Amundsen 2000). As was the case in a previous study of zebra finches (de Kogel & Prijs 1996), female bill colour in this experiment was not associated with condition once significance levels had been corrected for multiple comparisons. Nor did my males prefer redder-billed females, which is consistent with previous studies of male preferences for female bill colour (Burley & Coopersmith 1987; de Kogel & Prijs 1996). However, I also found no evidence that males preferred intermediate bill colours, which previously they have been shown to prefer (Burley & Coopersmith 1987). Male preferences for female bill colours may in part be age-dependent (see chapter 5). Furthermore, males are thought to imprint on the bill colours of their mothers (Vos 1995), which is liable to confound the use of condition signalling by females. Nevertheless, the relationship between female bill colour and fecundity is worthy of further experimental study.

The interaction between intrasexual competition and mate choice has received scant attention in socially monogamous species (Qvarnström & Forsgren 1998). This is surprising because dominant individuals may not always be preferred as mates, especially when environmental conditions or life history traits necessitate a trade-off between dominance and, for example, parental care. Furthermore, studies have generally concentrated on male-male competition and female choice. In many species with biparental care both male choice and female-female competition, and their interaction, may operate.

Chapter 3

Pair bond stability in the zebra finch is not influenced by the availability of an alternative mate in better nutritional condition.

ABSTRACT

Experimental investigations of the factors that influence pair bond stability in socially monogamous animals are relatively few. Here I examine the extent to which the availability of a mate with apparently higher reproductive capacity influences the mate preferences of pair-bonded zebra finches, *Taeniopygia guttata*. Unpaired birds show a strong preference for mates in better body condition. Birds whose breeding event was manipulated to fail with their established partner were presented with a choice between this partner and a novel bird whose previous diet had been manipulated to enhance reproductive performance. Despite the history of breeding failure, overall birds showed a clear preference for their established partner. In males, the preference for the established partner was strongest if she was relatively young. I discuss why the pair bond appears to be stable even in the face of an apparently better option in the zebra finch.

INTRODUCTION

Numerous animals exhibit preferences for mates possessing particular characteristics (Bateson 1983; Kirkpatrick & Ryan 1991; Andersson 1994). Preferences can be for a single copulation event or can result in a partnership during one or more breeding events, which usually involves co-operation between a male and a female to rear offspring (Wickler & Seibt 1983; Gowaty 1996). Social monogamy such as this is thought to be the mating system of over 90% of avian species (Black 1996). These social attachments are of variable length and are generally referred to as pair bonds. Long-term pair bonds that persist for more than a single breeding season (perennial monogamy) are less common, but occur in 21% of bird families (Black 1996).

Mate choice, however, can be thought of as a process that continues throughout life (Black 1996). Pair members may develop preferences for alternative mates and this will lower pair bond stability. This instability increases the likelihood of extra-pair copulation (EPC) or divorce, or both. The benefits and costs of both fidelity and mate change determine whether or not birds remain faithful (Birkhead & Møller 1992; Ens *et al.* 1996; McNamara & Forslund 1996). One clear benefit of mate change is the acquisition of a higher-quality mate (Choudhury 1995) and pair members often respond to alternative mating opportunities. An individual may, for example, remain in a socially monogamous pair bond but be sexually promiscuous by engaging in EPC (reviewed in Westneat *et al.* 1990). When mate familiarity improves reproductive success this should select for the maintenance of long-term pair bonds (Ens *et al.* 1996). Sometimes however, the benefits of remaining with an established mate may be outweighed by the benefits of changing mates, resulting in divorce (the better option hypothesis: Ens *et al.* 1993; also see, for example, Baeyens 1981; Davies 1989). Divorce and EPC can thus be considered extensions of the initial mate choice process, in which individuals re-assess the quality of their partner relative to other available mating opportunities (Choudhury 1995; Black 1996) (figure 1.1). This form of divorce is distinct from divorces not directly involving mate choice such as those produced by the intrusion of a third individual (forced divorce: Taborsky & Taborsky 1999), accidental loss (Owen *et al.* 1988) or as a side effect of differential arrival to breeding grounds (Choudhury 1995; Ens *et al.* 1996).

Most evidence that divorce can result from the choices of particular individuals is correlative (Smith 1991; Ens *et al.* 1993; Dhondt & Adriaensen 1994; Orell *et al.* 1994; Catry *et al.* 1997; Ramsay *et al.* 2000; Streif & Rasa 2001). Only one experimental study to my knowledge has demonstrated divorce for a better mating option (Otter & Ratcliffe 1996). It is generally assumed that females initiate divorce (Cezilly *et al.* 2000; Dhondt 2002), but in species with mutual mate choice and in which both sexes vary in quality, initiation of divorce should be more likely by the sex that is rarer (Cezilly *et al.* 2000). Both sexes therefore can be expected to initiate divorce when this carries fitness benefits (for example, Baeyens 1981; Matthysen 1990). In species exhibiting biparental care individuals should prefer mates with good parenting abilities (Reynolds & Gross 1990; Ryan 1997), which may be indicated by nutritional condition (Price *et al.* 1993).

Zebra finches *Taeniopygia guttata* exhibit mutual mate choice (Wynn & Price 1993) and show continuous, year-round partnerships and strong pair bonds (Butterfield 1970; Zann 1996). Extra-pair paternity has been found in 8% of broods in the wild (Birkhead *et al.* 1990) and 11% of broods in captivity (Birkhead *et al.* 1989). Divorce has not been recorded in wild populations (Zann 1996), although it has been observed in captivity (Morris 1954). In this species the nutritional state of females influences their egg laying capacity, and unpaired male zebra finches prefer females in good condition (Monaghan *et al.* 1996; Selman & Houston 1996; Jones *et al.* 2001). Females also have been shown to prefer males in good nutritional condition (Wynn & Price 1993), from whom they may receive direct benefits during a breeding event (Price *et al.* 1993). Manipulation of condition therefore provides a potential means of altering attractiveness experimentally. However, the extent to which these preferences may be modulated once birds enter a pair bond has not been studied.

In this study I examine whether the preference for an established mate in zebra finches is influenced by the availability of an alternative with apparently higher reproductive potential (stages 2 & 3, figure 1.1). To create this difference between potential reproductive performance with the established mate and the alternative I induced failure of the breeding attempts of experimental pairs and enhanced the nutritional state of the alternative partner.

METHODS

Experimental birds

Experimental birds were aged between 16 months and 36 months and had bred previously with a different partner. They were maintained on a standard-protein (SP) diet of *ad lib* mixed seed (foreign finch mixture; J. E. Haith, Cleethorpes, UK) and water, plus 5g of conditioning food (which comprised Rearing and Conditioning supplement (J. E. Haith), moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had previously been diluted 1g/litre with water) on two days per week. The temperature in the breeding room ranged between 19.5°C and 24.3°C. I simulated breeding failure in 25 experimental pairs by replacing all eggs, on the day that they were laid, with dummy eggs. This was done throughout the breeding period, including any replacement eggs that were produced, in order to simulate persistent failure.

Experimental pairs were kept in breeding cages (120cm x 45cm x 40cm) that contained two breeding pairs, separated by a perspex divider down the centre of the cage. Pairs were able to view each other through the divider. Each experimental pair manipulated to fail was always housed in visual contact with a control pair that was allowed to breed successfully. This served to inform the experimental pair that brood failure was not an unavoidable result of prevailing environmental conditions. I alternated the side of the cage (i.e. right or left) in which experimental and control pairs were housed. I recorded latency to lay and clutch size for each pair. Body mass was measured by using an electronic balance (± 0.1 g) when birds were paired initially and at the time of the mate preference tests.

Control pairs each reared at least one fledgling and experimental pairs were manipulated to remain unsuccessful for as long as it took their neighbouring pair to raise young. Therefore experimental pairs generally undertook prolonged incubation periods. Experimental pairs incubated the dummy eggs for an average of 39.6 ± 2.9 days (n = 25) compared to an average incubation period of control pairs of 14.6 ± 2.0 days (n = 25). Experimental pairs either abandoned incubation eventually or nest boxes were removed 4-6 days prior to the start of mate preference tests. Pair members remained together until the start of the mate preference tests.

Mate preference tests

In the mate preference tests the experimental birds were offered a choice between their established partner and an alternative mate. The alternative mate in a preference test comprised a novel bird either receiving the same SP diet as the established partner or receiving a high protein (HP) diet for four weeks prior to the preference tests. The HP diet was the same as that known to increase both the number and size of eggs laid by females and female attractiveness (Monaghan *et al.* 1996; Selman & Houston 1996; Jones *et al.* 2001). The HP diet consisted of *ad lib* mixed seed and water, plus approximately 5g of a mixture of conditioning food (see above) & homogenised hen's egg per cage per day. The alternative mating options were housed in unisex groups of 4 or 5 birds prior to the tests (cage dimensions: 120cm x 45cm x 40cm). Breeding birds had not previously seen these novel birds. Experimental females were on average 28.9 ± 6.4% lighter than HP novel females at the time of the preference tests, but were of a similar mass to SP novel females (table 3.1). However, there were no differences in body mass between experimental males and either of the two groups of novel males with which they were paired in preference tests (table 3.1). Experimental males and females that were presented with the two types of alternative option did not differ in their body mass (females: HP male option (n = 13) = 18.9 ± 0.9 g; SP male option (n = 11) = 18.1 ± 0.9 g; $t = 0.62$, $P = 0.541$; males: HP female option (n = 13) = 21.3 ± 1.0 g; SP female option (n = 11) = 21.6 ± 0.9 g; $t = 0.17$, $P = 0.869$) or age (females: HP male option (n = 13) = 27.4 ± 2.1 months; SP male option (n = 11) = 29.8 ± 2.3 months; $t = 0.79$, $P = 0.440$; males: HP female option (n = 13) = 25.2 ± 2.3 months; SP female option (n = 11) = 28.4 ± 2.8 months; $t = 0.88$, $P = 0.388$).

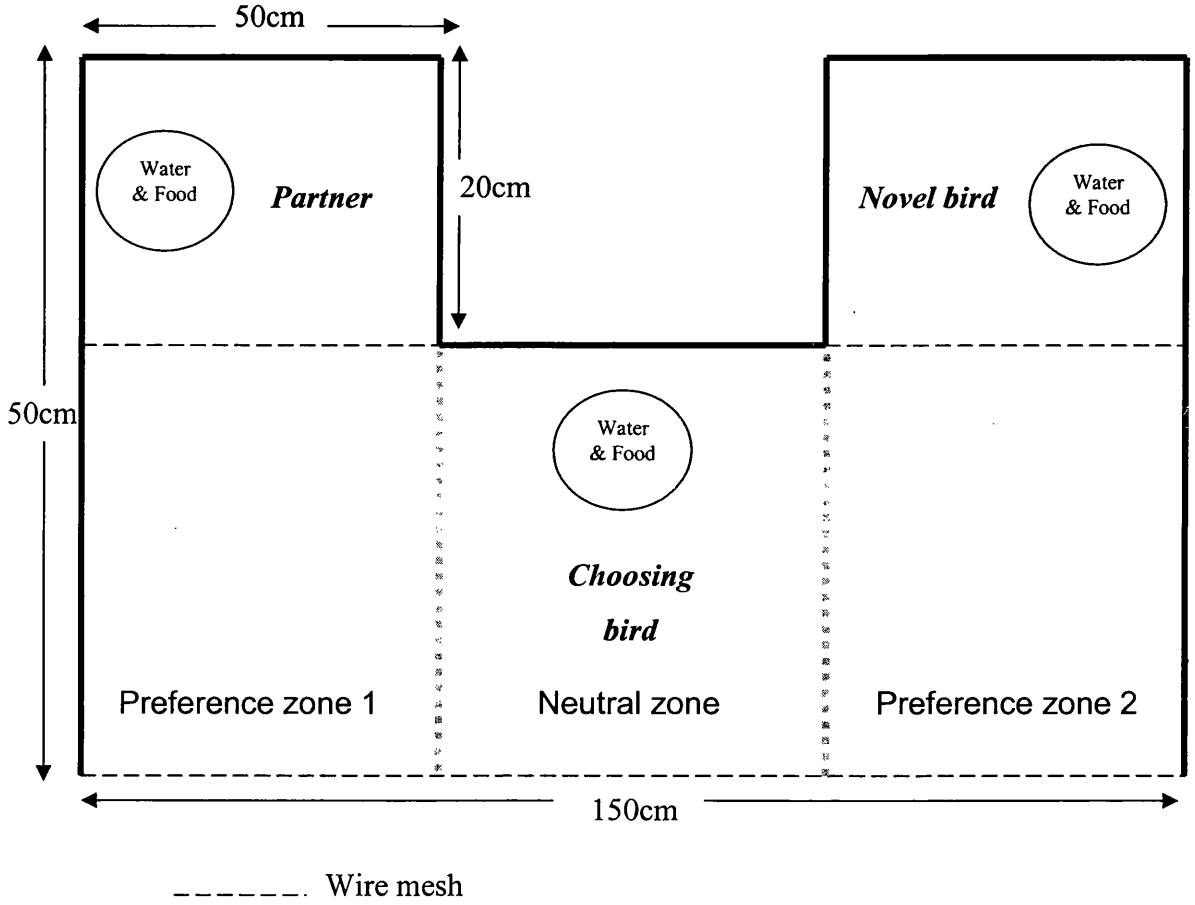
Mate preference tests lasted for three hours. All three birds were placed in a dichotomous-choice arena (figure 3.1), and allowed to acclimate for 10 minutes. They were then video recorded for 180 minutes, with the two mating options (the established partner and the novel bird) spending 90 minutes in each of two individual compartments on either side of the test arena. The positions of the established partner and the novel bird were exchanged halfway through the preference test. The dichotomous choice cage consisted of two preference zones either side of a central neutral zone. In order to see and interact with one of the two birds in their separate compartments, the chooser needed to enter one of the preference zones. Videos were

coded and watched blind with respect to the birds' identity several weeks later. The absolute amount of time the chooser spent in the two preference zones was recorded from the videos. Two birds, one of either sex, did not use the mate preference zones during the tests and thus could not be included in the analyses. Experimental birds spent a similar amount of the test time in the preference zones when faced with SP and HP novel birds (females: novel SP males (n = 11) = 51.08 ± 7.93 %, versus novel HP males (n = 13) = 56.08 ± 7.61 %, $t = 0.45$, $P = 0.655$; males: novel SP females (n = 11) = 80.53 ± 6.97 %, versus novel HP females (n = 13) = 74.03 ± 8.25%, $t = 0.59$, $P = 0.561$). These percentages of the total test times were labelled as the "active choice" times of birds. The percentage of active choice time in which the chooser associated with a bird was considered a measure of mate preference for that individual. Time spent affiliating with an individual has been validated as a measure of sexual attraction in zebra finches, as it is correlated with female tail quivering (ten Cate & Mug 1984) and predicts subsequent pair formation for both sexes (Clayton 1990).

Table 3.1 A comparison of the body mass of experimental birds and the novel birds with which they were entered into a mate preference test. Mean values (± standard error) of body mass at the time of the preference tests are provided. The experimental birds had each undertaken an unsuccessful breeding attempt with their established partner. In the preference test their partner was allowed to choose between them and the novel bird, which had been receiving either a high protein (HP) or standard protein (SP) diet. Tests were all paired *t*-tests.

Experimental bird	Mass (g)	Novel bird	Mass (g)	n	*t*	*P*
Females	18.4 (0.7)	HP	23.4 (0.8)	13	5.48	**<0.001**
Females	18.5 (1.0)	SP	17.2 (0.9)	11	0.94	0.369
Males	20.4 (0.8)	HP	22.7 (1.6)	13	1.20	0.252
Males	21.2 (1.1)	SP	23.0 (0.8)	11	1.55	0.152

Figure 3.1 Mate preference test arena. Experimental birds were required to choose between their established partner and a novel bird. The compartments housing the partner and the novel alternative both contained two perches and both the preference zones of the choosing bird contained a set of perch arrays. The positions of the partner and the novel bird were exchanged halfway through each preference test (see Methods). All birds had used the choice arena prior to breeding.



Statistical analysis

Analyses were carried out using SPSS (version 12.0 2004). All percentage values were arcsine square root transformed prior to analyses. The latency to lay data were left-skewed and were therefore log-transformed prior to analyses. Associations between mate preferences and mate quality variables were tested using models containing the diet of the novel bird (either HP or SP) as a fixed factor and the percentage of active choice time spent with the established partner as the dependent variable. Age can influence breeding performance (Newton 1989; Forslund & Pärt 1995) and mate preferences (for example, Kokko & Lindström 1996). As a consequence it is important to control for age in analyses of divorce (Black 1996; Ens *et al.* 1996). In this study experimental birds were aged approximately 16, 24 or 36 months at the time of the preference tests. Novel birds were all approximately 19 months old. The age of the choosing bird and the age of the established partner were entered into the models as covariates. Relative mass (mass of the established partner minus the mass of novel bird, as a percentage of the partner's mass), clutch size and latency to lay were also entered as covariates. Experimental birds may react differently to the alternative mating options depending on their age and their partner's age. Therefore I included in the initial models interactions between the diet of the novel bird and the chooser's age, diet and the partner's age and between the chooser's age and the partner's age. This model was run separately for males and females. Stepwise backward deletion of variables with non-significant effects was used, starting with non-significant interactions.

A separate ANCOVA model was used to assess whether any variable exerted different influences on male and female mate preferences. Sex was entered as a fixed factor, pair identity as a random factor and interactions between sex and covariates (see above) were included in the initial model. Stepwise backward deletion of interactions with non-significant effects was used.

RESULTS

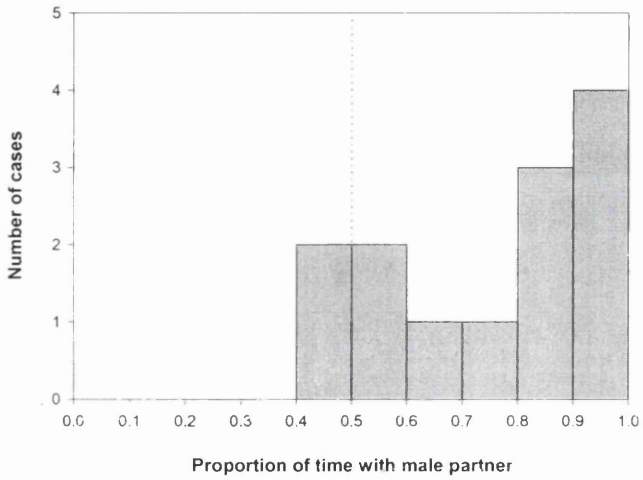
A majority of females preferred their established partner rather than the novel alternative, regardless of whether the alternative male was from an HP (two-tailed binomial test: $P = 0.022$) or SP ($P = 0.012$) nutritional background (figure 3.2). Although in 12.5% of cases (3 of 24 tests) females spent more time with the alternative mate. Variation existed in female mate preferences (mean percentage of active choice time spent with male partner = 76.80 ± 4.28 %, n = 24, CV = 27.32), but none of the measured variables explained a significant amount of this variation (table 3.2a).

Most males also preferred their established partner rather than the HP novel females ($P = 0.003$) or SP novel females ($P = 0.065$) (figure 3.3). However, males did prefer to spend time with the alternative mate in 16.7% of cases (4 of 24 tests). Male mate preferences also varied considerably (mean percentage of active choice time spent with female partner = 66.44 ± 4.19 %, n = 24, coefficient of variation (CV) = 30.86). The nutritional status of the novel female did not explain variation in the amount of active choice time males spent with that female (table 3.2b). Male mate preference was, however, affected by the age of the established partner (table 3.2b). Males spent relatively more time with their established partner if she was relatively young (figure 3.4). Clutch size laid during the breeding event with the established partner did not, however, differ between female age groups (females aged approximately 16 months: 4.25 ± 1.65, n = 4; 24 months: 5.67 ± 1.13, n = 9; 36 months: 4.18 ± 0.64, n = 11; ANOVA: $F_{2, 21} = 0.75$, $P = 0.486$).

The age of the established partner affected male and female mate preference differently (ANCOVA: sex: $F_{1, 42.8} = 3.82$, $P = 0.057$; interaction between sex and age of established partner: $F_{2, 43.2} = 3.61$, $P = 0.036$; pair identity: Wald $Z = 0.51$, $P = 0.612$). Male preference for an established partner decreased with the partner's age, whereas there was a non-significant trend for females to spend more time with their established partner if he was older (figure 3.5). All other interaction terms between sex and covariates were non-significant ($P > 0.251$).

Figure 3.2 Frequency distribution of the time experimental females spent with their male partner. Each female and her partner had undertaken a single unsuccessful breeding attempt. Females were presented with an alternative option to their established partner which was either a) a novel male previously provided with a high protein diet, or b) a novel male previously fed a standard protein diet (see Methods for details of the diets). The dotted line indicates the point of no preference for either male.

(a)



(b)

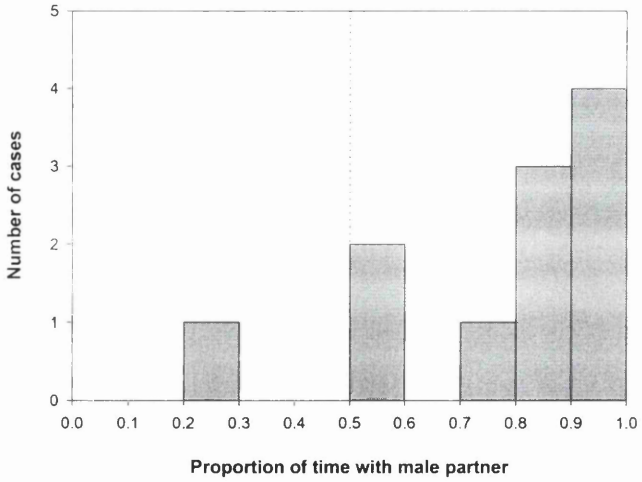
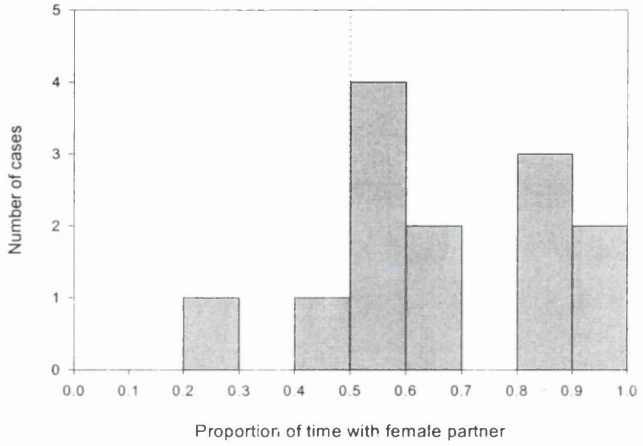


Figure 3.3 Frequency distribution of the time experimental males spent with their female partner. Each male and his partner had undertaken a single unsuccessful breeding attempt. Males were presented with an alternative option to their established partner which was either (a) a novel female previously provided with a high protein diet, or (b) a novel female previously fed a standard protein diet (see Methods for details of the diets). The dotted line indicates the point of no preference for either female.

(a)



(b)

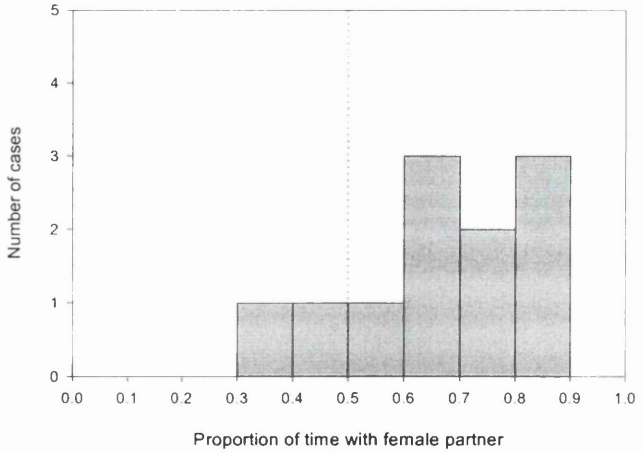


Figure 3.4 The relationship between mate preferences and the age of established partners. Birds were provided with a choice between their established partner, with whom they had completed a failed breeding event, and a novel alternative. Established partners were aged approximately 16, 24 or 36 months old. Males spent a greater percentage of active choice time (see Methods) with their established partner when she was relatively young. There was a non-significant trend for females to show a greater preference for their established partner if he was relatively old. The numbers of birds in each age category are shown in brackets. The novel birds were aged approximately 19 months.

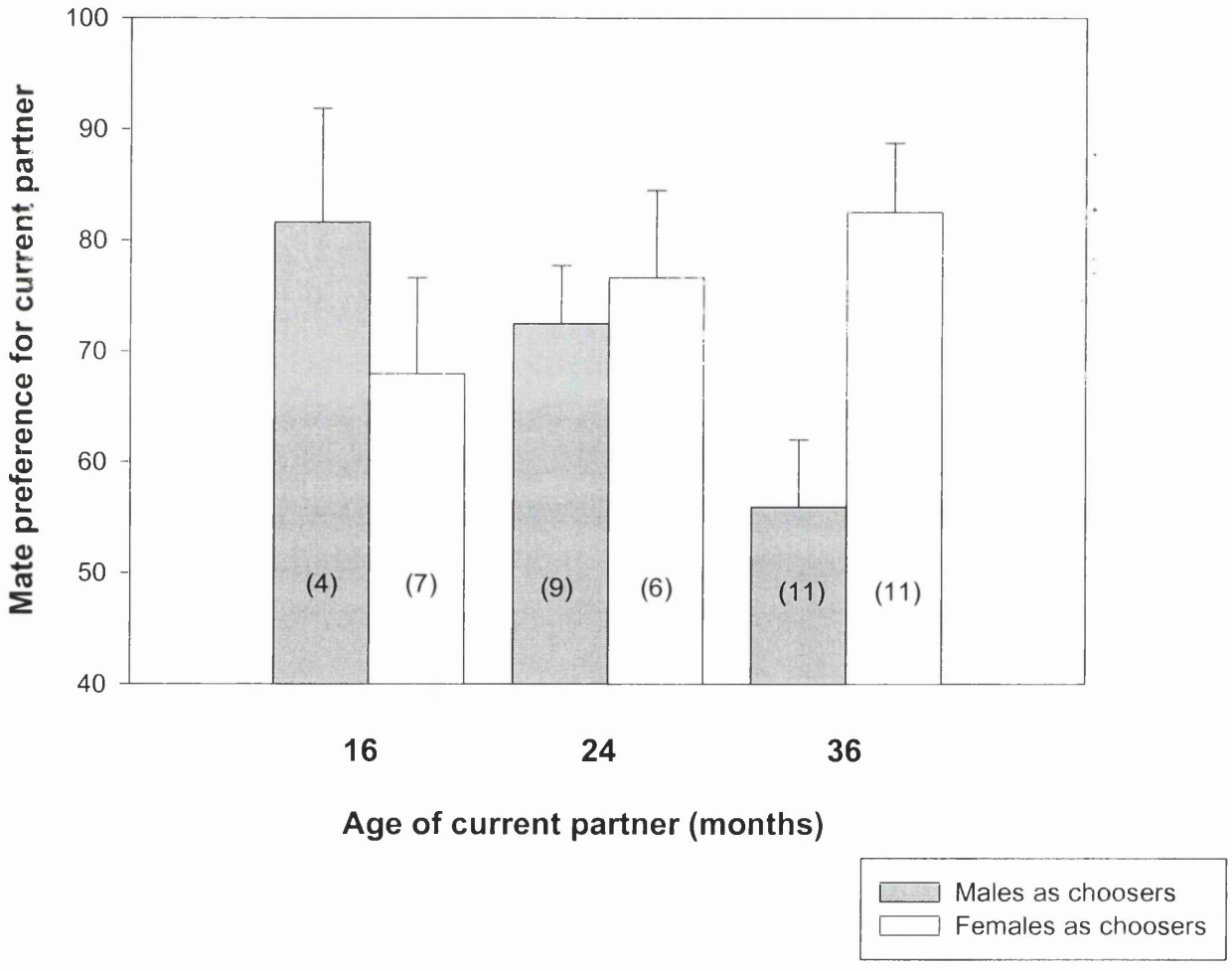


Table 3.2 Variables affecting (a) female and (b) male mate preference. Experimental birds were offered a choice between their established partner and a novel alternative mate. This novel alternative had received either a high protein (HP) or standard protein (SP) diet. All interaction terms were non-significant (P > 0.159).

(a) Female mate preference

Variable	*df*	*F*	*P*
Diet of the novel male	1, 17	0.25	0.626
Age of female	1, 18	0.56	0.464
Age of established partner	1, 22	1.51	0.232
Relative mass of partner & novel bird	1, 19	2.13	0.161
Clutch size	1, 20	2.11	0.162
Latency to lay	1, 21	2.11	0.161

(b) Male mate preference

Variable	*df*	*F*	*P*
Age of established partner	1, 22	8.19	**0.009**
Diet of the novel female	1, 21	0.24	0.630
Age of male	1, 19	0.40	0.535
Relative mass of partner & novel bird	1, 20	0.52	0.478
Clutch size	1, 18	0.15	0.704
Latency to lay	1, 17	0.07	0.794

DISCUSSION

Birds whose breeding attempt had failed with their established partner generally exhibited a preference for this mate, even if the alternative mate was in better nutritional condition than their partner was. Individuals in superior nutritional condition are likely to have enhanced reproductive potential (Monaghan *et al.* 1996; Selman & Houston 1996) but this did not override the preference for the established partner. This experiment therefore did not provide any evidence that the availability of an apparently better option reduces pair bond stability in the zebra finch.

My results indicate that prior pairing can modulate the preferences for mates in better body condition exhibited by unpaired individuals (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001). Unpaired males prefer females that are fed on diets similar to the HP diet provided in this study (Monaghan *et al.* 1996; Jones *et al.* 2001). However, in our population, paired males generally preferred their established partner, despite failing to breed with her and even though an alternative mate in better body condition was available. Unpaired females also prefer mates in a good nutritional state (Wynn & Price 1993). However, the HP diet treatment in this study did not create a noticeable difference in condition between the experimental and HP alternative males. As such I cannot be confident that HP alternative males represented better mating options from the perspective of the experimental females.

Despite birds generally preferring established partners, there was variation in the extent of interest shown by both sexes in alternative mates (figures 3.3 & 3.4). Indeed in 14.6% of tests the experimental bird preferred to affiliate with the alternative mating option rather than the established mate. Variation in male preferences can partly be explained by the relative age of the established partner. Previous zebra finch studies found no effects of age on reproductive performance (Zann 1994) or only limited effects at an early age (Williams & Christians 2003). It is generally assumed, however, that there is a quadratic relationship between age and reproductive performance. Performance may improve with age, but will probably decline in old age because of senescence (Newton 1989; Forslund & Pärt 1995). The younger experimental females in this study may therefore have had a high reproductive potential relative to the oldest (approximately 36 months old) experimental females. However, I found no evidence that clutch size changed with

age. Variation in female preferences could not be explained by any measured variable, but it may be related to unmeasured male attributes such as bill colour and display rate. Interestingly, females and males reacted differently to the age of their partner (figure 3.4).

These results indicate that pairs containing a relatively old female have low pair bond stability. Therefore an increased probability of EPC, divorce or both (as EPC and divorce may constitute a single, two-step, mate-sampling strategy: Colwell & Oring 1989; Heg *et al.* 1993; Cezilly & Nager 1995) in these pairs can be predicted. However, my results provide no evidence that the availability of an alternative mate with apparently higher reproductive potential weakens pair bond stability in the zebra finch.

Why is the zebra finch pair bond particularly stable? One possibility is that mate familiarity improves breeding performance in this species and thus represents a benefit of pair fidelity and a cost of divorce. Pair bond duration has been found to improve breeding performance in several species, possibly due to an improvement in pair co-ordination over time (Black 1996; Ens *et al.* 1996). When the benefits of fidelity are high, large differences in quality between an established mate and an alternative mate are likely to be required, *ceteris paribus*, in order for an individual to gain from divorce. Although previous studies found no effects or only limited effects of age on reproductive performance, and no effect of breeding experience (Zann 1994; Williams & Christians 2003), no studies have explicitly tested whether pair bond duration (i.e. mate familiarity) affects performance. The possibility that fidelity benefits pairs by improving reproductive performance via mate familiarity effects therefore cannot be excluded.

Year-round pair bonds place limitations on the scope for mate change in zebra finches. The requirement of this species to breed opportunistically, during rare periods of rainfall, necessitates constant physical closeness, and a constant state of physiological readiness (Zann 1996). Zebra finch pairs are generally inseparable and maintain close physical contact, except when incubating and brooding (Zann 1996). Divorce may therefore entail intrasexual competition costs for the third party involved (i.e. the alternative mate) (stage 4, figure 1.1). The maintenance of physical closeness and physiological readiness requires reproductive hormonal adjustments. When separated from a partner, zebra finches show elevated levels of corticosterone (CORT) (Remage-Healey *et al.* 2003), which return to baseline levels upon reunion.

Elevated CORT levels during even a brief period of separation may suppress the formation of new mate preferences. This is liable to protract the process of re-pairing and thereby help to maintain an existing pair bond. This idea has support from studies of socially monogamous mammals. CORT levels have been found to decline in unpaired female prairie voles *Microtus ochrogaster* when encountering a novel male. Furthermore, experimental adrenalectomy sped up the establishment of a mate preference, whereas experimentally increased CORT levels hindered the formation of new mate preferences (DeVries *et al.* 1995).

One factor that may influence the outcome of preference tests is the time available for assessment. In this study, the available non-partner birds had not been seen by the experimental birds prior to the experiment, and the test duration was relatively short (three hours). The assessment time available to individuals will determine how accurately mate quality can be evaluated (Sullivan 1994). If a longer period was available over which to assess the alternative mating options then the effect on the mate preferences of paired birds, and therefore pair bond stability, may change. Zebra finches are naturally colonial and gregarious. In the wild therefore, it is possible to assess alternative mating options within a colony over a long time period, which may provide opportunities to evaluate the breeding performance of other birds. Nevertheless, my results fit the pattern of species that exhibit year-round pair bonds having low divorce rates (Ens *et al.* 1996). Alternative mating options with apparently superior phenotypes may not be preferred to an established mate if there are large benefits to pair fidelity.

Chapter 4

Reproductive performance and pair bond stability: an experimental test

ABSTRACT

Mate choice is a dynamic process that can continue even after the formation of social partnerships. Individuals in a pair bond will often re-assess their established partners relative to alternative mating options. Mate preferences for these alternative options can lead to either extra-pair copulations or divorce. One factor that individuals can use in partner assessment is breeding success with that partner relative to that of other simultaneously breeding pairs. Here I investigate experimentally whether relative success affects the mate preferences of pair-bonded birds in the zebra finch, *Taeniopygia guttata*. Pairs whose breeding events were manipulated to fail were each in visual contact with a neighbouring pair that was allowed to breed successfully. I found that most birds, regardless of relative breeding success, spent more time with their established partner in preference tests, rather than their neighbour of the opposite sex. However, among females, but not males, the level of interest in the alternative partner (i.e. their neighbour) was higher in those that had experienced breeding failure, when controlling for the effects of mass and age. These results therefore provide evidence that breeding performance affects pair bond stability in the zebra finch. Furthermore, both females and males showed a higher level of interest in their neighbour when this alternative mate was heavy in relation to their established partner. Females also showed higher interest levels in the alternative mate when this bird was older than their established partner.

INTRODUCTION

The length of association between reproductive partners can vary. Birds are unusual among animal taxa because in many cases they have long-lasting partnerships. Long-term, socially monogamous pair bonds that persist for more than a single breeding season occur in 21% of bird families (Black 1996). The maintenance of these long-term bonds can have considerable fitness benefits (Ens *et al.* 1996). However, pair members continuously re-assess the quality of their established partner (Black 1996) and may develop a preference for an alternative mate, thereby reducing the stability of the pair bond. This can lead to a pair member changing mates, either by maintaining the social pair bond and engaging in extra-pair copulations (EPCs) (reviewed in Westneat *et al.* 1990) or by severing the bond and divorcing their established partner (reviewed in Choudhury 1995). One factor that a pair member may consider when re-assessing the quality of their established partner is their own prior reproductive performance with that partner relative to other pairs simultaneously breeding in the same environmental conditions. There is correlative evidence suggesting that reproductive failure encourages divorce (reviewed in Dubois & Cézilly 2002). However, as birds age they have a reduced tendency to divorce (McNamara & Forslund 1996), but their reproductive success increases (Ens *et al.* 1996). Consequently the effects of breeding success and age on divorce are often confounded (Coulson & Thomas 1983; Orell *et al.* 1994; Ens *et al.* 1996; Sydeman *et al.* 1996).

Three experimental studies have addressed the question of whether breeding performance affects divorce rate by using brood size manipulations. In great tits, *Parus major*, divorce rate was lower among pairs whose breeding success was experimentally increased than in pairs whose breeding success was reduced (Lindén 1991). In another two studies, an experimental reduction in breeding success had no significant effect on divorce rate (Orell *et al.* 1994; Blondel *et al.* 2000). Divorce rates were 47%, 22% and 73% for pairs of blue tits, *Parus caeruleus*, with reduced broods, controls and pairs with enlarged broods respectively (Blondel *et al.* 2000). It is unclear, however, whether the absolute number of young or the proportion of their hatchlings that are fledged is more important for the perception of breeding success. Pairs of blue tits whose brood size was reduced in number actually fledged a greater proportion of their hatchlings, even though in absolute terms, they fledged fewer

offspring than pairs whose brood size was enlarged (Blondel *et al.* 1998). Alternative experimental manipulations such as the simulation of hatching failure would circumvent this type of ambiguity.

The fitness benefits of changing mates versus maintaining a pair bond may also vary with the sex of the bird. Female initiation of divorce is generally inferred from observations such as females experiencing greater success than males subsequent to mate change or females being less likely to remain unpaired than males (Jones & Montgomerie 1991; Dhondt & Adriaensen 1994; Orell *et al.* 1994; Desrochers & Magrath 1996; Hannon & Martin 1996; Catry *et al.* 1997; Streif & Rasa 2001). Most studies have thus concluded that it is the females who usually control pair bond maintenance and dissolution in birds (Cézilly *et al.* 2000; Dhondt 2002; but see Baeyens 1981; Matthysen 1990). Yet in species with mutual mate choice and in which both sexes vary in quality, the potential fitness gains from mate change should be similar for males and females, suggesting either sex may initiate divorce (McNamara *et al.* 1999; Cézilly *et al.* 2000).

In this study I examined pair bond strength in relation to reproductive success by experimentally inducing breeding failure in captive zebra finches, *Taeniopygia guttata*. The zebra finch is a perennially socially monogamous species exhibiting mutual mate choice (Wynn & Price 1993; Zann 1996). Extra-pair paternity has been found in 8% of broods in the wild (Birkhead *et al.* 1990) and 11% of broods in captivity (Birkhead *et al.* 1989). Divorce has been observed in captivity in this species (Morris 1954), though not in the wild (Zann 1996). Each experimental pair with a failed breeding attempt was neighboured by, and was therefore in visual contact with, one other successfully breeding pair. I used mate preference tests to measure pair bond stability, as reflected by interest in an established partner relative to an alternative partner (i.e. a neighbour of the opposite sex). I compared the interest of successful males and females in their established partners with the interest of experimentally-failed breeders in their partners.

METHODS

Manipulation of reproductive success

Experimental birds were all housed in a breeding room that was maintained at a temperature of 21.25 ± 0.08°C. Each cage (120cm x 45cm x 40cm) contained two breeding pairs. Birds were maintained on a diet of *ad lib* mixed seed (foreign finch mixture; J. E. Haith, Cleethorpes, UK) and water, plus 5g of conditioning food (which comprised Rearing and Conditioning supplement (J. E. Haith), moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had previously been diluted 1g/litre with water) on two days per week. Birds aged between 16 and 36 months and with previous breeding experience prior to the experiment were randomly paired and assigned to either a reproductively successful or unsuccessful treatment group. There was no difference between the groups in the age of males ($t_{50} = 0.16$, $P = 0.872$), the age of females ($t_{50} = 0.89$, $P = 0.380$), the body mass of males at the start of the experiment ($t_{50} = 1.66$, $P = 0.103$), female body mass at the start of the experiment ($t_{50} = 1.29$, $P = 0.202$), clutch size ($U = 282.50$, $n_1 = 27$, $n_2 = 25$, $P = 0.299$) or latency to lay ($U = 253.50$, $n_1 = 27$, $n_2 = 25$, $P = 0.123$).

The pairs in the successful group were allowed to breed and rear chicks to independence. Breeding failure in the unsuccessful group was created by removing any eggs that pairs laid, and replacing them with dummy eggs. This was done throughout the breeding period, including the removal of any replacement eggs that were produced, in order to simulate persistent failure. Successful and unsuccessful pairs were housed in the same cage, separated by a perspex divider down the centre of the cage, so that pairs were able to view each other and had information on their relative breeding success. This arrangement informed unsuccessful pairs that brood failure was not a result of prevailing environmental conditions and also served to allow experimental birds to familiarise themselves with their neighbours, whom they would later encounter in mate preference tests at the end of breeding. I alternated the side of the cage (i.e. right or left) in which successful pairs were housed. Thirty-two pairs successfully reared at least one fledgling, but in 5 cases one of the pair members died during the raising of the offspring. Among the 32 unsuccessful neighbouring

pairs, in 3 cases one of the pair members died prior to the preference tests; 4 unsuccessful pairs failed to lay eggs and they were not entered into the tests as choosers since there was no evidence that they had in fact formed pair bonds. They were, however, used as alternative mating options for their successful neighbours. Birds from 27 successful and 25 unsuccessful pairs were therefore entered into preference tests as choosers.

I recorded latency to lay and clutch size for each pair. Body mass was measured by using an electronic balance (± 0.1g) when birds were paired initially and at the time of mate preference tests. Successful pairs (n = 27) reared 2.44 ± 1.28 young that were removed at independence (day 35). Whenever these pairs failed to hatch any young from their clutch (n = 4) they were given at least one chick within 2 days of hatching in order to simulate success. Unsuccessful pairs (n = 25) incubated dummy eggs for an extended period (39.6 ± 2.9 days versus 14.6 ± 2.0 days in successful pairs). Dummy eggs were removed at the same time that neighbouring fledglings were removed.

Mate preference tests

Pairs remained together for 5 days after young or dummy eggs were removed and were then entered into mate preference tests. Each pair member was entered into a test, in which they were presented with their established partner and their neighbour of the opposite sex. Birds were placed in a dichotomous-choice arena (figure 3.1). This consisted of two preference zones either side of a central neutral zone. In order to see and interact with one of the two birds (i.e. either the established partner or neighbour), the chooser needed to enter one of the preference zones. Birds were allowed to acclimate for 10 minutes and were then video recorded for 90 minutes. The positions of the partner and the neighbour were then exchanged and birds were allowed to re-acclimate for 10 minutes, prior to another 90-minute recording session. Mate preference tests therefore lasted for three hours in total, excluding acclimation periods. Videos were watched without knowledge of the status of the birds several weeks later and I recorded the absolute amount of time each chooser spent in each of the two preference zones. Some members of the 27 successful pairs and 25 unsuccessful pairs could not be used as choosers because a neighbouring pair member had died (n = 3 successful males, 3 unsuccessful males and 2 unsuccessful females).

Preference tests were conducted, however, when a widowed neighbour was available. In addition, two choosers failed to use the preference zones in the test arena (n = 1 successful female and 1 unsuccessful female). As a result, data from mate preference tests were obtained for 22 unsuccessful male, 22 unsuccessful female, 24 successful male and 26 successful female choosers.

Birds spent the majority of their time in the preference zones, rather than in the central neutral zone (unsuccessful females: 60.98 ± 4.67 %, n = 22; successful females: 67.14 ± 5.21 %, n = 26; unsuccessful males: 67.67 ± 5.07 %, n = 22; successful males: 70.46 ± 5.10 %, n = 24). These times in the preference zones were labelled the "active choice" times of birds and did not differ between sex or experimental group (sex: $F_{1, 90} = 0.98$, $P = 0.325$; breeding success: $F_{1, 90} = 0.78$, $P = 0.379$; sex by breeding interaction: $F_{1, 90} = 0.11$, $P = 0.740$). The percentage of active choice time spent with one or other of the two birds was considered to be a measure of the level of interest in that individual as a potential sexual partner (ten Cate & Mug 1984; Clayton 1990). A bird was judged to be preferred as a mate when the chooser spent more than 50% of its active choice time with this individual. Pair bond strength was measured as the proportion of active choice time spent with the established partner; the greater the proportion, the stronger the pair bond.

Statistical analysis

Analyses were carried out using SPSS (version 12.0, 2004). All percentage and proportion values were arcsine square root transformed prior to analyses. The latency to lay data were left-skewed and were therefore log-transformed prior to analyses. The appropriate non-parametric tests were used when data did not meet the assumptions of parametric tests. I tested associations between the percentage of active choice time spent with an established partner and breeding performance (either unsuccessful or successful) as a fixed main effect. In a separate analysis I tested whether males and females differed in their mate preference response, with respect to breeding success. In this analysis, sex was entered as a main effect. Age and body mass can influence mate preference (Kokko & Lindström 1996; Monaghan *et al.* 1996; Jones *et al.* 2001). Therefore relative age (the age of the established partner minus the age of the neighbour) and relative mass (mass of the established partner minus the mass of the neighbour, as a percentage of the partner's mass) were included in the analyses as

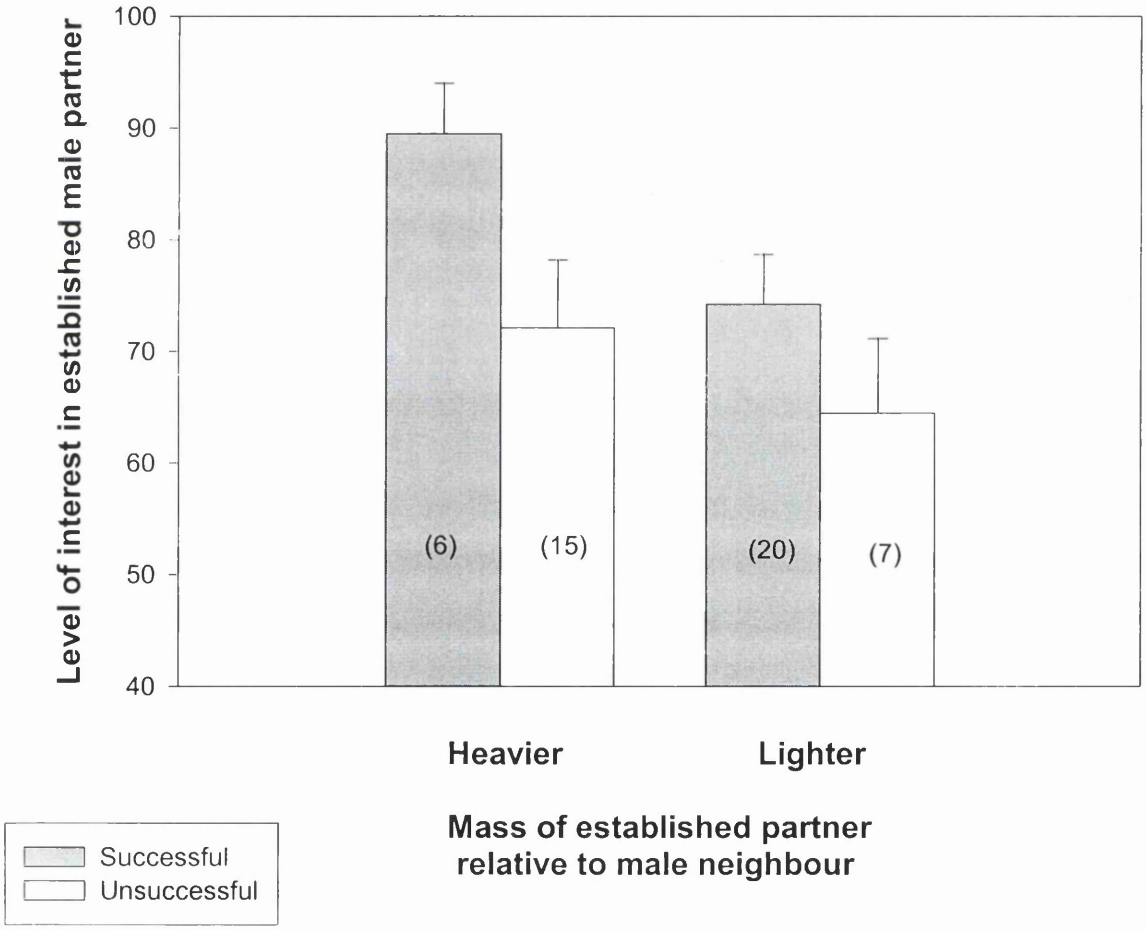
covariates. The age, clutch size and latency to lay of the chooser were also entered as covariates because these traits varied and may have influenced mate preference. Cage identity was entered into each model as a random factor to control for the fact that the same two mating options (i.e. same-sex neighbours) were generally used in two separate tests in which their respective partners were the choosers. An unsuccessful pair and a successful pair that had visual contact with each other were given the same cage identity. Models were run first separately for males and females and then for both sexes combined. This second model included the identity of established pairs as an additional random factor nested within cage identity. In addition, both analyses were run with the exclusion of (1) successful pairs that reared only foster chicks (n = 4), (2) successful pairs whose neighbours had failed to lay eggs (n = 4) and (3) birds whose preferences were tested with a widowed neighbour (n = 3 successful females, 3 unsuccessful females and 2 unsuccessful males). These additional models gave qualitatively the same results as the full models (analyses not shown). Interactions between main effects and covariates were included in the initial models and stepwise backward elimination of non-significant ($P > 0.05$) interactions and then non-significant main effects was used. Means ± standard errors are presented.

RESULTS

The proportion of active choice time spent by females with their established partner varied appreciably (74.04 ± 2.97 % (n = 48), coefficient of variation (CV) = 27.75). Breeding performance with the established partner explained a significant amount of the variation in female interest levels in their established partner (table 4.1a); unsuccessful females spent more time with their neighbouring male than did successful females when controlling for differences in body mass and age between the two options. Furthermore, females showed less interest in their established partner when he was lighter (figure 4.1a) or younger (figure 4.2) than the alternative option. Breeding success, the relative mass of an established partner and a partner's age each explained more than 20% of the variation in female mating preferences (table 4.1a). Female interest levels were not affected by her own age, clutch size or latency to lay (table 4.1a). The interaction between female age and breeding success bordered on

Figure 4.1 Percentage of active choice time (see text) spent with an established partner during a mate preference test, in relation to breeding performance and the partner's mass relative to a neighbour (i.e. an alternative mating option). Females (a) spent less time with their established partner if it was lighter than the male neighbour (when statistically controlling for breeding success, see table 1a). Males (b) spent less time with their partner when it was lighter than their female neighbour (also see table 1b). Analysis was performed with actual mass difference as a covariate; these "heavier" and "lighter" categories are used only for ease of presentation. Brackets contain the number of birds present in each category.

a) Female mate preference



b) Male mate preference

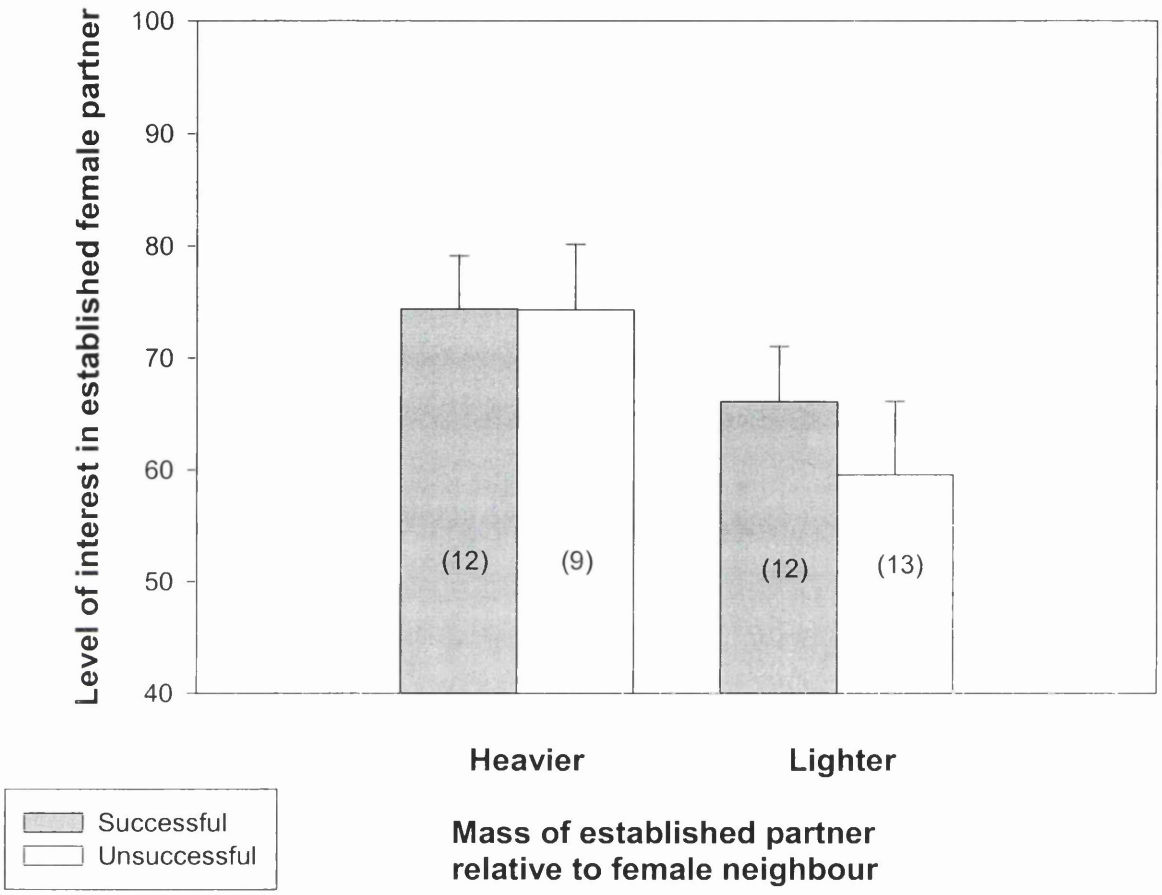
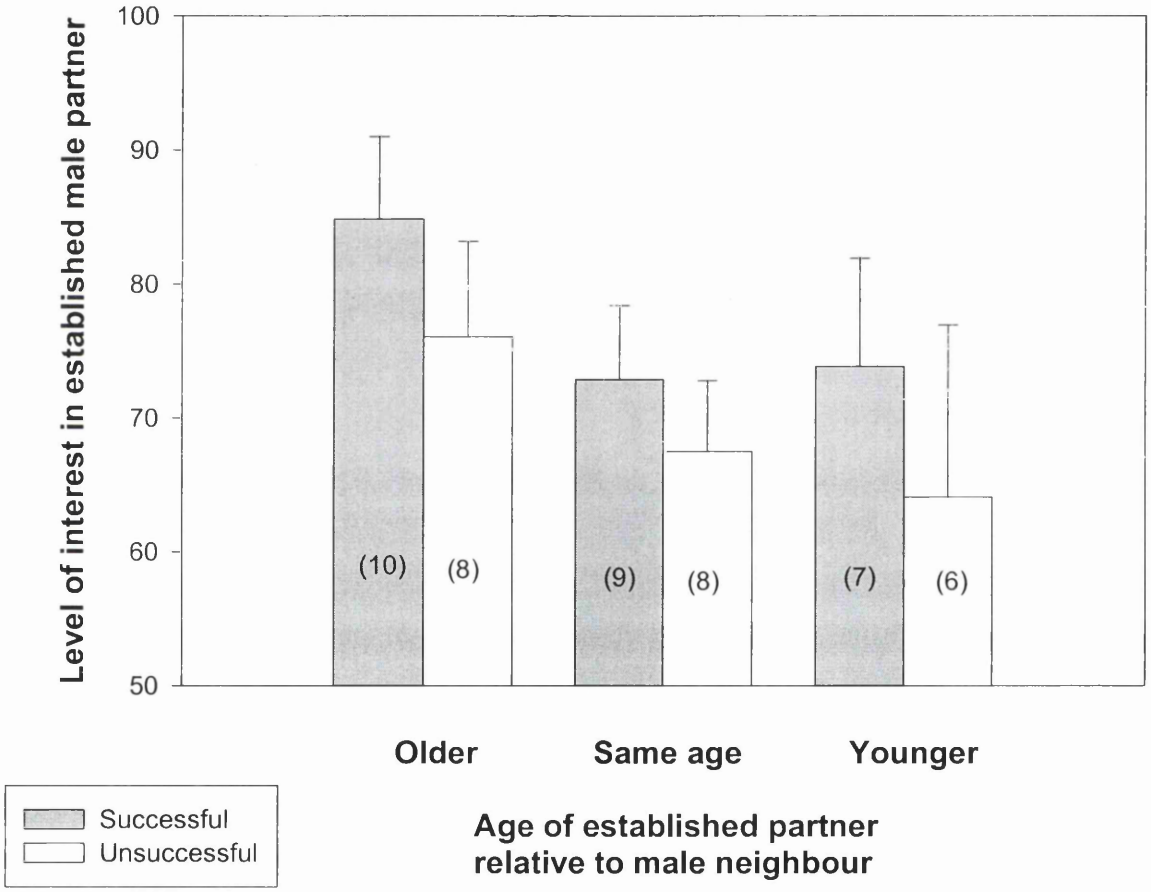


Figure 4.2 Percentage of active choice time (see text) spent by experimental females with an established partner during mate preference tests, in relation to breeding performance and the partner's age relative to a neighbour (i.e. an alternative mating option). The established partner was older than, approximately (i.e. a difference of less than one month) the same age as, or younger than the male neighbour. Female preference for her established partner was reduced if her established partner was younger than the alternative option (see table 1a). Analysis was performed with age difference as a covariate; these categories are used only for ease of presentation. Brackets contain the number of birds present in each category.



significance (table 4.1a), suggesting that the reaction of a female to her breeding performance with her established partner could be influenced by her age. However, most females, regardless of breeding performance, preferred their established partner rather than the alternative mating option (two-tailed binomial tests: 19/22 unsuccessful females: $P = 0.001$, mean percentage of active choice time with partner = 69.67 ± 4.63%; 24/26 successful females: $P < 0.001$, mean = 77.74 ± 3.75%; figure 4.3a).

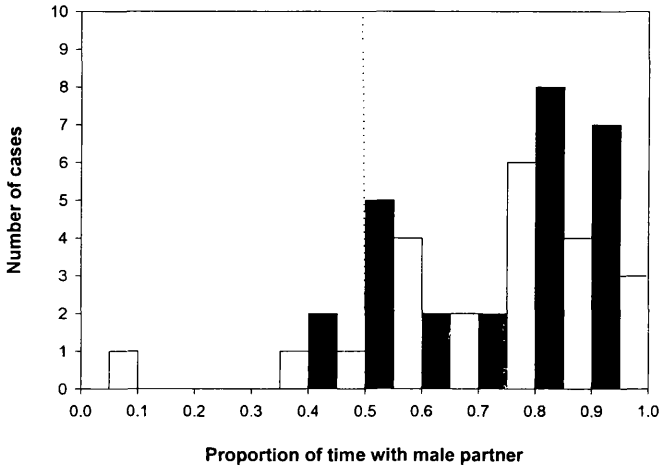
There was considerable variation in the proportion of active choice time spent by males with their established partner (68.02 ± 2.88 % (n = 46), CV = 28.74). The level of interest shown by males in their established partner was higher when the established partner was heavier than the alternative option (figure 4.1b). Success with the established partner had no effect on male interest levels in the alternative options (table 4.1b). Nor were male interest levels affected by their own age, clutch size, latency to lay or the relative age of their established partner (table 4.1b). The majority of males, irrespective of breeding success, spent more time with their established partner than their female neighbour (two-tailed binomial tests: 17/22 unsuccessful males: $P = 0.017$, mean = 65.60 ± 4.73%; 21/24 successful males: $P < 0.001$, mean = 70.25 ± 3.46%; figure 4.3b).

Males and females responded differently to the presence of an alternative option that was older than their established partner (mixed model: cage identity: Wald $Z = 0.12$, $P = 0.903$; Pair identity (nested within cage identity): Wald $Z = 1.46$, $P = 0.144$; sex: $F_{1, 46.0} = 3.39$, $P = 0.072$; age difference between the mating options: $F_{1, 62.3} = 0.06$, $P = 0.813$; interaction between sex and age difference between the options: $F_{1, 58.6} = 4.40$, $P = 0.040$). Females spent more time with their established partner, when controlling for breeding success and mass, when he was older than the neighbouring male (figure 4.2 and table 4.1a), whereas males did not discriminate between females based on age (table 4.1b). Males and females did not respond differently to breeding success (breeding success: $F_{1, 27.6} = 1.32$, $P = 0.260$; interaction between breeding success and sex: $F_{1, 48.6} = 1.31$, $P = 0.257$; all other interaction terms: $P > 0.235$).

Experimental pairs from the successful group differed in their hatching success, their absolute number of fledglings, the proportion of their clutch that fledged and the proportion of their hatchlings that fledged. However, none of these measures were associated with the proportion of active choice time that successful pair

Figure 4.3 Frequency distribution of the time (a) experimental females and (b) experimental males spent with their established partner during mate preference tests. Each experimental bird and their partner had undertaken a single breeding attempt. Unsuccessful birds (open bars) were presented with a successful neighbour of the opposite sex as an alternative option to their partner. Successful birds (closed bars) were presented with an unsuccessful neighbour of the opposite sex as an alternative option. The dotted line indicates the point of no preference for either option.

a) Female mate preference



b) Male mate preference

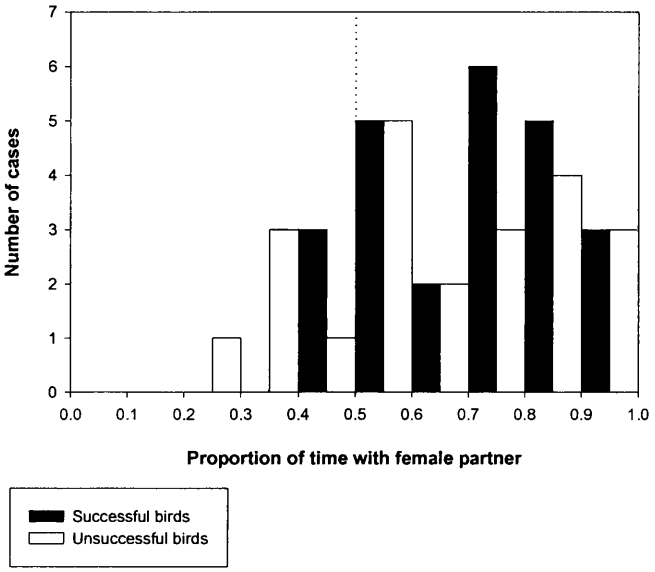


Table 4.1 Variables affecting (a) female (n = 48) and (b) male (n = 46) mate preference. Experimental birds had undertaken a single breeding attempt and were offered a choice between their established partner and a neighbouring bird of the opposite sex. Unsuccessful birds were presented with a successful neighbour as an alternative mating option to their partner. Successful birds were presented with an unsuccessful neighbour as an alternative mating option. The dependent variable in each mixed model was the percentage of active choice time spent with an established partner. (a) Cage identity (random factor): Wald $Z = 0.36$, $P = 0.717$, parameter estimate = 0.005 ± 0.014; all interaction terms were non-significant (although the interaction between the age of the choosing female and breeding success bordered on significance: $P = 0.053$); (b) Cage identity (random factor): Wald $Z = 1.29$, $P = 0.197$, parameter estimate = 0.014 ± 0.011; all interaction terms were non-significant (P > 0.070).

(a) Female mate preference

Variable	*df*	*F*	*P*	Parameter estimate (± SE)	η^2
Breeding success	1, 23.0	7.99	**0.010**	0.230 (0.081)	0.26
Age difference between partner & neighbour	1, 22.9	7.07	**0.014**	0.009 (0.003)	0.24
Mass difference between partner & neighbour	1, 26.5	6.68	**0.016**	0.213 (0.082)	0.20
Age of female	1, 41.3	0.07	0.789	0.007 (0.003)	
Clutch size	1, 37.7	0.22	0.642	0.009 (0.018)	
Latency to lay	1, 34.6	< 0.01	0.973	-0.005 (0.158)	

(b) Male mate preference

Variable	*df*	*F*	*P*	Parameter estimate (± SE)	η^2
Mass difference between partner & neighbour	1, 22.9	7.57	**0.011**	0.174 (0.063)	0.25
Breeding success	1, 22.2	0.26	0.617	0.030 (0.059)	
Age difference between partner & neighbour	1, 20.8	1.24	0.279	-0.003 (0.003)	
Age of male	1, 35.2	0.01	0.917	0.004 (0.007)	
Clutch size	1, 37.2	2.15	0.151	-0.027 (0.018)	
Latency to lay	1, 35.8	0.01	0.925	0.012 (0.007)	

members spent with their established partner during preference testing (females: $r_s <$ 0.33, $P > 0.100$; males: $r_s < 0.37$, $P > 0.075$).

DISCUSSION

The majority of birds, regardless of sex and breeding performance, spent more time with their established partner than with their neighbour of the opposite sex. This underscores the fact that the zebra finch pair bond is generally very stable (Zann 1996). As discussed in chapter 3, the effect of pair bond duration on breeding performance in the zebra finch has yet to be tested, therefore the importance of social mate fidelity in this species is unknown. However, considerable variation existed in the levels of interest shown in alternative options, and therefore in pair bond stability. I found that this variation in pair bond stability could in part be explained by the combined influence of breeding success, relative body mass and age.

The level of interest exhibited by both males and females in an alternative mate was lower when their established partner was heavier than the alternative option. Relative body mass and condition has been shown to be a mate choice cue used by both sexes in the zebra finch (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001). Interestingly, I found that body mass had no effect on pair bond stability when the alternative options consisted of novel birds that had not been seen before (chapter 3). In the experiment described in chapter 3, birds were only able to assess the novel options during a 3-hour test. During the current experiment, however, birds were able to observe their alternative options for several weeks while breeding. The accuracy of mate quality assessment should improve when the duration of the assessment period is increased (Sullivan 1994). Choosing birds in this experiment may have been able to assess the relative body condition of their partner and the alternative option with more accuracy than they were able to when presented with a novel alternative option.

The level of interest shown by females in an alternative option, when statistically controlling for mass and breeding success, was higher when this alternative was older than her established partner. As little is known about age-related mate choice or age-related changes in breeding performance in zebra finches, it is not

possible to pinpoint why females in this experiment exhibited more interest in older rather than younger alternative mates (although see chapter 5). Nevertheless, older males are expected to be preferred as mates when age is associated with parental ability (Forslund & Pärt 1995), genetic quality (Kokko & Lindström 1996) or the signalling of an attractive trait (Kokko 1997). In contrast, I did not find that male test behaviour was influenced by female age. There was a trend, however, for males to spend more time with younger established partners than they spent with older established partners, which reinforces the results of tests with novel alternative mates (chapter 3).

Breeding success affected the level of interest in alternative mates exhibited by females, but not males, when body mass and age were statistically controlled. Thus female interest in alternative males appears to be influenced by the additive effects of relative breeding success, male age and male body mass. This suggests females assess whether males represent better mating options based on a combination of both past reproductive success and phenotype. Lifjeld & Slagsvold (1988) found a similar pattern in their study of pied flycatchers, *Ficedula hypoleuca*. Female pied flycatchers divorced their partner following breeding failure, but only if there was a more attractive mating option available.

Given that male and female zebra finches invest similar amounts of effort in breeding events (Zann 1996), it is surprising that breeding performance should affect the mate preferences of females but not males. My results suggest that pair-bonded males may be influenced to a greater extent by the phenotype of their partner in relation to the phenotypes of alternative mates, rather than by relative breeding success. However, the absence of population-level mating preferences may often mask underlying preferences held by subsets of the population (Basolo 2004). Indeed, my results suggest that the way a female reacts to her breeding performance with an established partner may partly be influenced by her age (interaction between female age and breeding performance: $P = 0.053$), but this would require further study. Certain types of males may also have reacted differently to breeding failure than others and additional experiments would be needed to investigate whether this is the case. Alternatively, females and males may respond differently to various aspects of breeding performance such as clutch size, hatching success (which was the experimental manipulation used in this study), fledging success and the behaviour of

their partner during parental care. However, no evidence of this was found in this study.

Reduced pair bond stability increases the likelihood of either EPC or divorce, or both, but it is not possible in this study to discriminate between these possibilities. It has been suggested that EPC and divorce may form a two-step process, representing a single mate-sampling strategy (Colwell & Oring 1989; Heg *et al.* 1993; Cézilly & Nager 1995). Overall 13.8% of birds (n = 94) showed more interest in alternative mates than in established partners. Interestingly this is similar to the level of extra-pair paternity recorded in this species (Birkhead *et al.* 1989; Birkhead *et al.* 1990). Divorce has not been studied experimentally in zebra finches and has not been observed in the wild (Zann 1996), but it has been observed in domesticated birds (Morris 1954)[1].

Many previous studies have found a correlative relationship between breeding performance and pair bond stability (reviewed in Dubois & Cézilly 2002). However, in the majority of cases, unsuccessful pairs do not divorce (Ens *et al.* 1996). For divorce to occur in species possessing year-round partnerships, such as zebra finches, the costs of intrasexual competition must be overcome (stage 4, figure 1, chapter 1). Hormones such as corticosterone may also limit the scope for re-pairing in socially monogamous animals (Remage-Healey *et al.* 2003). Moreover, divorce is only likely to be a profitable strategy when a better mating option than the established partner is available (McNamara *et al.* 1999). Of the birds that have experienced poor breeding performance with their partner, only a proportion will have a better option available to them. Nevertheless, mate quality and reproductive success will often be correlated in the wild and therefore alternative mating options will generally be required to exceed only a low quality threshold in order for an unsuccessful bird to initiate divorce (McNamara *et al.* 1999). However, in this experiment I uncoupled mate quality and breeding performance by randomly assigning birds to the breeding manipulations. This probably meant that an even smaller pool of unsuccessful birds had a better quality mating option available to them compared to the situation in a natural setting. Several experimental birds may have failed with an apparently high quality partner and this may provide another explanation for why so few unsuccessful birds preferred successful alternative mates.

[1] One case of divorce was observed and described in this study, but no divorce rate data were provided.

In species with relatively large clutch sizes, using a dichotomous measurement of success (successful or unsuccessful) may underestimate the effect of breeding performance on pair bond stability if it is more appropriate to consider success as a continuous variable (Dubois & Cézilly 2002). For example, divorce rates did not differ between pairs of blackbirds, *Turdus merula*, labelled successful (1, 2 or 3 fledglings) or unsuccessful (zero fledglings), but did decline with the number of fledglings produced (Desrochers & Magrath 1996). Zebra finches lay relatively large clutches (i.e. a modal clutch size of 5 (Zann 1996); 4.85 ± 0.28 in this study) and, for example, fledging a single chick from five eggs may not be perceived as a success by some pairs. My criterion for success in this study was that a pair should raise at least one chick to independence, and successful pairs varied in the proportion of their clutch that they fledged (17 to 100%). Therefore the true effect size of breeding performance on the mate preferences of pair-bonded birds may have been masked. However, I did not find that this was the case because pair bond stability among successful pairs was not related to any measure of breeding performance.

Breeding success is one cue that paired animals can use when re-assessing an established partner. However, although the stability of a pair bond may often be affected by prior reproductive performance, subsequent mate change decisions are likely to be dependent on the nature of the other mating options that are available to an individual. The importance of breeding performance on the stability of pair bonds is likely to be determined by species-specific ecological and life history traits (Ens *et al.* 1996; Dubois & Cézilly 2002).

Chapter 5

Discrimination between naïve and competent mates in the zebra finch.

ABSTRACT

In socially monogamous taxa, which include a substantial number of bird species, age and experience are factors that may influence mate selection. Here I provide evidence that competent mates (i.e. relatively old birds with previous breeding experience) are preferred over naïve mates (i.e. relatively young birds without breeding experience) by both male and female zebra finches, *Taeniopygia guttata*, in preference tests. However, this preference was only found amongst relatively old female choosers and relatively active male choosers. The mate preferences of the oldest male choosers, but not relatively young male choosers, also appeared to be influenced by female bill colour. Relatively old, experienced males preferred females with bill hue in the middle of the phenotypic range. The competent and naïve mating options did not differ in any of the attributes that I measured and it is therefore unclear how birds discriminated between these potential mates. These results suggest that the importance of age and breeding experience as mate choice cues for socially monogamous animals may vary between birds, depending on their own prior experience and choosiness.

INTRODUCTION

Some animals form social partnerships in which they jointly raise offspring. These pair bonds are particularly common amongst birds (Black 1996). In such socially monogamous species, mate preferences are expected to favour mates that provide good parental care and resources, thus supplying choosing individuals with direct fitness benefits (Reynolds & Gross 1990; Ryan 1997). One factor that may influence the quality of care and resources a potential mate can offer is their previous breeding experience, which is generally correlated with age. Indeed, older birds often exhibit better reproductive success than younger birds (Newton 1989). As birds gain breeding experience, they may increase their efficiency in tasks such as incubation, feeding and brooding (Forslund & Pärt 1995). The experience that older birds have accumulated should also give them an advantage in locating and competing for resources (Forslund & Pärt 1995). Even when the influence of experience is minimal, breeding success may increase with age if older birds optimise their reproductive effort towards the end of their lives (Williams 1966), or if low-quality phenotypes gradually disappear (reviewed in Forslund & Pärt 1995). Older animals may also be genetically superior to relatively young individuals (Kokko & Lindström 1996; Kokko 1998; but see Hansen & Price 1995).

Mating preferences can often vary between individuals within the same population (Wagner 1998; Widemo & Sæther 1999; Forstmeier & Birkhead 2004), especially when these individuals differ in age, breeding experience or body condition (i.e. condition-dependent choice: Jennions & Petrie 1997). Studies of population-level preferences alone may consequently overlook underlying preferences exhibited by subsets of a population (Basolo 2004). High-quality individuals are expected to be choosier than low-quality birds (i.e. assortative mating for quality: Burley 1977). Preference functions may also change with experience if animals are able to learn what represents a good mate; for example, male and female feral pigeons, *Columba livia*, prefer older mates with breeding experience, rather than younger, inexperienced mates, but this preference is expressed more consistently by experienced birds (Burley & Moran 1979).

Discrimination between experienced (i.e. relatively old) and inexperienced (i.e. relatively young) mates requires breeding experience (i.e. age) to be reliably

signalled (i.e. correlated with a phenotypic trait: Manning 1985). For example, individuals may increase their investment in advertising effort as they age (Kokko 1997; Proulx *et al.* 2002) or attractiveness and longevity may be correlated (i.e. selective early death of unattractive individuals) (Kokko 1998). Aspects of courtship proficiency, such as the co-ordination and polish of displays, may improve with sexual experience (Schubert *et al.* 1989).

Here I present the results of preference test experiments, conducted with zebra finches, *Taeniopygia guttata*, in which birds were offered a choice of a competent mate (i.e. an older bird with breeding experience) or a naïve mate (i.e. a younger bird without breeding experience). Age and experience are invariably associated in nature (Newton 1989) and I made no attempt to distinguish between the two in this experiment. Instead I examine birds from the two extremes of the continuum. The zebra finch is a socially monogamous species in which both males and females have strong mate preferences (Wynn & Price 1993). Zebra finches preferentially mate with respect to body condition (Wynn & Price 1993; Monaghan *et al.* 1996), bill colour (Burley & Coopersmith 1987), song rate (Collins *et al.* 1994) and song syntax (Clayton & Pröve 1989). Previously it has been observed that older males gain a mating advantage over younger males in this species (Schubert *et al.* 1989). I test here (1) whether population-level preferences of males and females are influenced by the previous breeding experience (and therefore age) of available mates, (2) whether breeding experience (and therefore age) is related to factors that are known to be involved in zebra finch mate selection and (3) whether variation in between-individual preferences can be explained by the attributes of the individual choosers.

METHODS

Experimental birds

The experimental birds that were labelled "competent" were approximately 4 years old at the time of the mate preference tests. This represented the oldest cohort in our stock population and included 17 males and 12 females. Domesticated zebra finches held under optimal breeding conditions can live for 5-7 years (Burley 1985) and are

often still capable of breeding when they are 5 or 6 years old (Immelmann 1965). These birds had bred on at least two occasions, most recently eight months prior to the mate preference tests. When not breeding they were housed in unisexual groups comprising 5-6 birds from their own age group (cage dimensions: 120 cm x 45 cm x 40 cm). For most of their lives they had received a diet of *ad lib* mixed seed (foreign finch mixture; J. E. Haith, Cleethorpes, UK), water, grit and cuttlefish, with approximately 0.75g of conditioning food (which comprised Rearing and Conditioning supplement (J. E. Haith), moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK) which itself had been diluted 1g/litre with water) per week per bird and sprouts and cress once per week.

The experimental birds that were labelled "naïve" (males: n = 17; females: n = 12) were approximately 8 or 9 months old at the time of the mate preference tests, at which age zebra finches are capable of breeding (Zann 1996). Experimental birds were selected randomly from a pool of 19 females and 21 males of this age. These birds were reared for the first 17 days of their lives on a diet of *ad lib* mixed seed and water, plus 5g of conditioning food (see above) per family on two days per week. Their post-fledging diet consisted of *ad lib* mixed seed, water, grit and cuttlefish, with approximately 0.75g of conditioning food once per week per bird and sprouts and cress once per week. This was the same diet that had been given to the experienced experimental birds for the previous 8 months. Inexperienced experimental birds remained in their original family groups until independence at day 35, when they were transferred to unisexual groups of 6 or 7 birds (cage dimensions: 120 cm x 45 cm x 40 cm). They remained in these groups until the mate preference tests. Young experimental birds therefore had no breeding experience prior to the preference tests. Young males were allowed to view and court non-experimental females through a wire mesh for two weeks prior to the tests as part of an investigation into sperm quality, but they were unable to mate. Young females had no courtship experience prior to the preference tests.

Biometry

The body mass of experimental birds was recorded by using an electronic balance (to the nearest 0.1 g), wing length using a wing rule (to the nearest 0.5 mm) and tarsus

length (to the nearest 0.1 mm) using a sliding calliper. Body size measurements were recorded 32.8 ± 0.8 (n = 58) days prior to the preference tests. Tarsus length was measured from the nuchal notch to the end of the tarsus with the foot flexed at a right angle. Wing length was measured from the wrist joint to the end of the longest primary feather. The body mass of all birds was recorded on the morning of the mate preference tests. Mass was corrected for structural size using a linear regression of mass on tarsus length and wing length. The standardised residuals derived from this regression were used to represent size-corrected mass, which provides an estimation of body condition (Piersma & Davidson 1991; Lindstrom & Piersma 1993).

Bill coloration was measured using both visual ranking (32.8 ± 0.8 (n = 58) days prior to the preference tests) and spectral analysis of bill reflectance (23.2 ± 0.8 (n = 58) days prior to the preference tests). Bill colour may be a condition-dependent indicator of male quality in the zebra finch used by females in mate choice (Birkhead *et al.* 1998; Blount *et al.* 2003; Burley & Coopersmith 1987). Males are thought to prefer females with bill colours in the middle of the phenotypic range (Burley & Coopersmith 1987). Bill colour was subjectively ranked on a scale ranging from 1 (light orange) to 9 (dark red) using colour chips (Dulux Trade Colour Palette; Dulux, UK). These chips covered the full range of bill colours exhibited by the experimental birds (see Appendix for full details of hue, chroma and brightness values). A subset of experimental birds was measured independently by two observers and between-observer repeatability was highly significant ($r_s = 0.89$, n = 27, $P < 0.001$).

The reflected radiance of bills was measured in daylight conditions using a fibre optic spectrometer (S2000 Fiber Optic Spectrometer, Ocean Optics, Florida, USA). Birds were held under a tungsten-halogen lamp, with their mandible rested in a wooden notch. The spectrometer probe was then placed centrally on the maxilla at a 45° angle and reflectance recorded in a computer database. Five measurements per bird were recorded and mean values at 0.35nm intervals calculated. I calculated hue (as in Smiseth *et al.* 2001) as the wavelength corresponding to median reflectance

$$\left(\lambda\left[\frac{R\max + R\min}{2}\right]\right)$$

Zebra finch bills reflect in the UV spectrum, as well as reflecting long wavelength light in the visible spectrum (figure 6.1). However, little is known about the precise role of UV signals in zebra finch mate preference formation (although see Bennett *et*

al. 1996; Hunt *et al.* 2001). Therefore analyses focussed on long wavelength visible light emissions, which appear to be more important than UV signals in zebra finch mate choice (Hunt *et al.* 2001). The bill reflectances of 7 (3 males and 4 females) of the 29 naïve birds were measured 7 days before a second observer measured the remainder of the birds. I controlled for observer in analyses of bill reflectance as observer significantly affected hue (n = 29; influence of observer on median reflectance: $F_{1, 26} = 37.15$, $P < 0.001$; sex: $F_{1, 26} = 25.54$, $P < 0.001$). Visual scores of bill coloration were associated with hue calculated as median reflectance (males (n = 34): $F_{1, 31} = 71.81$, $P < 0.001$, controlling for observer: $F_{1, 31} = 23.97$, $P < 0.001$; females (n = 24): $F_{1, 21} = 8.92$, $P = 0.007$, controlling for observer: $F_{1, 21} = 31.61$, $P < 0.001$). This suggests that subjective visual assessment of bill colour closely approximates with an objective measurement of bill colour. The correlation also indicates that visual assessments reflect the hue component of colour.

Song analysis

Song rate is thought to be a condition-dependent trait that reflects male quality (Birkhead *et al.* 1998). Several studies have found that female zebra finches prefer males with high song rates (ten Cate & Mug 1984; Houtman 1992; Collins *et al.* 1994; de Kogel & Prijs 1996). Indeed it has been suggested that song rate is a more important choice criterion than male bill colour (Collins & ten Cate 1996) even though the two are often correlated (Houtman 1992; de Kogel & Prijs 1996). Song rate in mate preference tests was calculated by analysing the number of song bouts (bout = a series of song phrases separated from other series by an interval of at least 5 seconds (Zann 1996)) produced by an experimental male within the initial 6 minutes after a female chooser began to affiliate with him. Whenever a female had spent more than 6 consecutive minutes with a male, subsequent song bouts were not included in song rate calculations. This took into account declines in song rate over time (for example, compare the amount of song produced over 1 minute and 5 minutes, table 5.1). Song rate was calculated as the number of song bouts divided by the number of relevant scan samples (see "Mate Preference tests" section). This value was averaged over the three tests to produce a single value for each male. However, this is a relatively inaccurate method of measuring song rate, as bouts will differ in the number of phrases they contain and birds will have different phrase lengths.

I also measured the song rate of experimental males (n = 29) 40.4 ± 0.5 days after the preference tests by recording song using an Aoi ECM-1025 microphone and Sony TCM-20DV recorder. In the intervening period four experienced males had to be culled as a result of an infection in their cage. A fifth experienced male was not included in the song rate recording session because of illness, but its song was recorded at a later date for syntactical analysis. One inexperienced male, despite singing during the preference tests, failed to sing in six subsequent recording sessions. Males were placed alone in a cage for 5 minutes, with the previously unseen female in an adjacent chamber. The mean proportion of time spent singing during both the first minute and the entire five-minute recording sessions were calculated. This was done by recording the number of phrases sung, and multiplying this by the mean phrase length (in seconds) to provide an estimate of the total amount of singing time. Song bout rate (log-transformed) during the preference tests was not significantly correlated with the proportion of time males spent singing independently of the tests (preference test song rate and singing time during the initial minute of the non-test recording: r_s = 0.29, n = 29, P = 0.133; test song rate and singing time during 5 minutes of non-test recording: r_s = 0.32, n = 29, P = 0.093). This suggests that males behaved differently in the two situations.

Song syntax was measured from sonograms (8.79 ± 1.06 phrases per male, using GW Instruments Soundscape package, GW Instruments, Inc., Massachusetts, USA). Zebra finches sing a repeated song phrase, which is stereotyped, and consists of subunits known as elements (or syllables) (Zann 1996). These elements are distinguished from each other by time gaps or amplitude changes. I recorded the number of different elements experimental birds had in their repertoires and the total number of elements contained in a full stereotyped phrase. These measures occasionally differed as some birds repeat elements within a phrase. Females have been shown to prefer males with the most complex songs (i.e. a large number of elements per phrase) in several species (Catchpole 1987) including the zebra finch (Clayton & Pröve 1989).

Mate preference tests

Sexually experienced birds, aged between 25 and 37 months old, were offered a choice between a competent experimental bird and a naïve bird. Each of these choosers had bred at least once before and had no prior contact with these mating options. Each test dyad (17 male dyads and 12 female dyads) comprised one competent and one naïve experimental bird. Competent and naïve birds did not differ in any traits other than age and experience (table 5.1). I matched birds by mass and visual assessment of bill colour into test dyads. There was no difference in body mass between naïve and competent birds within test dyads (paired *t*-tests: males: $t_{16} = 1.51$, P = 0.152; females: $t_{11} = 1.93$, P = 0.080). However, within the test dyads, naïve males had slightly redder bills (mean difference of 0.65 ± 0.24 on a 1-9 scale) than old males (Wilcoxon signed-rank test: $Z = 2.77$, $P = 0.006$), but there were no significant within-dyad differences in female bill colour (Wilcoxon signed-rank test: $Z = 1.73$, $P = 0.084$).

Preference tests were conducted in a dichotomous-choice arena (figure 3.1) under full spectrum, artificial light (Bird Lamp, Arcadia, Croydon, UK). Experimental birds were placed in test cages and the preference tests were started after an acclimation period of 15 minutes. The behaviour of the chooser was recorded from behind a screen, approximately 1.5 metres from the test cages. Every third minute the position (either (1) in the central neutral zone; (2) in the right-hand zone; or (3) in the left-hand zone) and behaviour ((1) feeding; (2) turned away from the experimental bird; (3) facing the experimental bird; (4) courting the experimental bird) of the chooser was recorded. Males were recorded as courting an experimental female if they produced head bows, bill wipes, directed song or hop-pivots (see Zann 1996). Females were judged to be engaged in courtship when they produced head twists and bows, hopping and tail-quivers (Zann 1996). Position and behaviour were recorded after 30 and 60 seconds within each observational minute. I also calculated a measurement of activity level: the proportion of active choice time during which a chooser courted or faced toward (i.e. showed interest in) a particular experimental bird. In addition, during testing of female test dyads, I recorded the number of song bouts directed to each female by choosing males. Each preference test lasted for 90 minutes; therefore 60 scan samples were recorded per test. The position recordings were then converted into an estimate of the amount of time spent with each bird.

Table 5.1 A comparison the traits of (a) naïve (n = 17 unless otherwise stated) and competent (n = 17 unless otherwise stated) experimental males and (b) naïve (n = 12) and competent (n = 12) experimental females. Tests were either *t*-tests or Mann-Whitney U tests (*Z* values) unless otherwise stated.

(a)

Trait	Naïve male	Competent male	Test statistics	*P*
Age	8 to 9 months	Approx. 48 months	-	-
Breeding attempts	0	At least 2	-	-
Mass (g)[a]	21.1 (0.5)	20.4 (0.7)	$t_{32} = 0.77$	0.446
Tarsus length (mm)[a]	15.1 (0.1)	15.1 (0.2)	$t_{32} = 0.25$	0.804
Wing length (mm)[a]	60.9 (0.4)	61.8 (0.4)	$t_{32} = 1.63$	0.113
Body condition[a]	0.12 (0.21)	-0.12 (0.26)	$t_{32} = 0.70$	0.489
Bill colour (visual score)[b]	6.50 (6.00, 7.00)	6.00 (5.00, 7.00)	$Z = 1.32$	0.186
Hue 1 (λ of median reflectance) (nm)[a]	599.48 (1.74)	593.79 (3.13)	$F_{1, 31} = 0.89$[c]	0.352
№ different elements in song repertoire[b]	5.00 (4.00, 5.00) (n = 16)	5.00 (4.00, 6.00) (n = 13)	$Z = 1.40$	0.162
Total № elements in full song phrase[b]	5.00 (4.00, 5.00) (n = 16)	6.00 (4.00, 7.00) (n = 13)	$Z = 1.58$	0.114
Song rate in mate preference tests (bouts min^{-1})[b]	0.33 (0.13, 0.94)	0.43 (0.17, 0.86)	$Z = 0.40$	0.692
Proportion of time singing in song assay (during initial minute)[b]	0.03 (0.00, 0.25)	0.14 (0.00, 0.25) (n = 12)	$Z = 0.33$	0.745
Proportion of time singing in song assay (during 5 minute period)[b]	0.03 (0.00, 0.11)	0.05 (0.01, 0.10) (n = 12)	$Z = 0.41$	0.686

(b)

Trait	Naïve female	Competent female	Test statistics	*P*
Age	8 to 9 months	Approx. 48 months	-	-
Breeding attempts	0	At least 2	-	-
Mass (g)[a]	20.7 (1.1)	19.7 (0.8)	$t_{22} = 0.76$	0.456
Tarsus (mm)[b]	15.6 (14.9, 16.5)	15.2 (14.8, 15.5)	$Z = 1.30$	0.194
Wing (mm)[a]	60.0 (0.5)	60.9 (0.5)	$t_{22} = 1.21$	0.239
Body condition[a]	0.08 (0.28)	-0.08 (0.28)	$t_{22} = 0.41$	0.685
Bill colour (visual score)[b]	3.25 (3.00, 4.50)	3.00 (2.63, 3.88)	$Z = 0.92$	0.360
Hue 1 (λ of median reflectance) (nm)[a]	591.51 (2.65)	583.61 (2.02)	$F_{1, 21} = 0.96$[c]	0.338

[a] Mean (± SE).

[b] Median (first and third quartiles).

[c] ANOVA, controlling for observer as an additional factor.

Each test dyad was entered into three tests with different choosers. Each female test dyad was presented to a different male chooser in each test (a total of 36 male choosers). Due to a shortage of suitable female choosers, some were used twice (n = 19), although with different test dyads (a total of 32 different female choosers). Males, on average, spent 94.15 ± 1.22 % and females 90.92 ± 1.69 % of the total test time with the experimental birds. These percentages were labelled as the active choice times of the choosing birds (defined as the sum of time spent with the two test options, excluding time spent in the neutral central zone; figure 3.1). The proportion of active choice time spent with each of the females was deemed to be an index of mating preference. A female was judged to be preferred as a mate when the male spent more than 50 % of his active choice time with her.

Statistical analysis

I analysed population-level preferences by averaging the behaviour of the three choosers per test dyad. Three "uninterested" female choosers were excluded as they were not recorded as facing toward or courting either of their male options. Preference

strength was measured as (1) the proportion of active choice time spent with a particular bird (i.e. affiliation behaviour), and (2) the proportion of active choice time during which a chooser was courting or showing interest in a particular bird (i.e. courtship behaviour).

I tested which factors influenced variation in chooser preferences by using mixed models containing the proportion of active choice time spent with the competent bird as the dependent variable and test dyad identity as a random factor. Each of the three choosers assigned to a particular test dyad received the same dyad identity. As some female choosers were used in more than one test, chooser identity was entered as a second random factor in the model of female preference. Chooser age (log-transformed) was entered as a covariate. The proportion of their active choice time that a chooser spent facing toward or courting the two mating options was entered as a measure of their overall "activity level". Within-dyad differences in body condition, bill hue and, in the model of female preference, preference test song rate were entered as covariates. These differences were calculated as the score of the competent male minus the score of the naïve male. I ran the model of male preference a second time using a different measure of relative bill colour. I calculated the deviation of each female's bill hue from the population median (586.92nm, n = 24 females). The within-dyad difference in deviation (naïve minus competent female) was entered as a covariate instead of hue difference, as males are expected to prefer females with bills of intermediate colour (Burley & Coopersmith 1987). Models were qualititatively unaltered when mass was entered rather than condition, visual scores were entered rather than hue, non-preference-test song rate was used and when the number of previous breeding attempts were entered rather than age (results not shown). Interactions between chooser age and activity level and other covariates were entered into both the initial models. I used stepwise backward elimination of non-significant effects in models of both male and female preferences, beginning with interactions.

Mixed models were run in SAS (version 8, SAS Systems 2001) and for all other analyses I used SPSS (version 10.0, 1999). Non-parametric tests were used in cases where data did not meet the assumptions of the equivalent parametric test. I checked that data were normally distributed using the Kolmogorov-Smirnov test and tested for equality of variances using Levene's test. All proportional data were arcsine square root transformed before analysis. Measures of song rate and activity level were

left skewed and were therefore log-transformed. All tests are two-tailed, means ± 1 standard error are presented unless otherwise stated.

Table 5.2 The behaviour in mate preference tests of sexually experienced (a) females and (b) males. These choosing birds were given a choice between a naïve (i.e. sexually mature, but inexperienced) experimental bird and a competent (i.e. experienced, older experimental bird). Data from three female choosers were not included in analyses (see Methods). All tests were paired *t*-tests that compared behaviour toward a naïve experimental bird and a competent experimental bird.

a)

Female Behaviour (n = 17)	**Naïve male (±SE)**	**Competent male (±SE)**	***t***	***P***
Affiliation behaviour[a]	40.58 (4.62)	59.42 (4.62)	1.80	0.092
Courtship behaviour[b]	9.51 (1.58)	15.31 (1.55)	2.65	**0.017**

b)

Male Behaviour (n =12)	**Naïve female (±SE)**	**Competent female (±SE)**	***t***	***P***
Affiliation behaviour[a]	42.32 (3.19)	57.68 (3.19)	2.40	**0.035**
Courtship behaviour[b]	16.19 (2.58)	22.59 (2.96)	1.16	0.269
№ song bouts[c]	39.17 (6.93)	38.50 (6.04)	0.21	0.835
Song rate[d]	0.68 (0.16)	0.37 (0.06)	1.45	0.176

[a] The percentage of active choice time (see Methods) that choosing birds spent with a particular opposite-sex bird.
[b] The percentage of active choice time during which the chooser was either courting or facing toward a particular experimental bird.
[c] The total number of song bouts directed at a particular female in a mate preference test.
[d] The number of song bouts per minute, during time spent with a female.

RESULTS

Female choosers were presented with naïve and competent males in preference tests. Females preferred to affiliate with competent males in a greater number of test dyads than those in which they preferred to affiliate with naïve males (two-tailed binomial test, 12 competent males preferred, 3 naïve males preferred, 2 ties not included, $P = 0.035$). Females, however, did not spend a greater proportion of their test time with

competent males than with naïve males, but they were more active with the competent males (table 5.2a). The affiliation behaviour of females during tests was influenced by their previous breeding experience (and therefore age) (table 5.3a). Relatively old females preferred competent males, whereas relatively young females showed no preference for either naïve or competent males (figure 5.1). Test behaviour was unaffected by either female activity levels or the phenotypic traits of the male mating options (table 5.3a).

Male choosers were presented with naïve and competent females in preference tests. There was no difference in the number of naïve and competent birds that were preferred in the preference tests (9 competent females preferred, 3 naïve females preferred, $P = 0.146$). Nevertheless, on average, males spent a significantly greater proportion of their active choice time with competent females (table 5.2b). The activity levels of males with competent and naïve females were, however, not different (table 5.2b). Male affiliation behaviour depended on their level of activity in the tests (table 5.3b). Relatively active males, but not relatively inactive males, spent a greater proportion of their time with the competent female rather than the naïve female (figure 5.2). Older males also appeared to discriminate between females based on bill colour when controlling for dyad identity and male activity level (table 5.3b). Older males tended to prefer bills of intermediate hues, whereas younger males were not generally influenced by female bill hue (figure 5.3). Male chooser age and activity level were not related ($r_s = 0.02$, n = 36, $P = 0.926$).

DISCUSSION

Older males that had bred previously and were thus reproductively competent were preferred as mates rather than reproductively naïve males, but only by females that were relatively old and experienced themselves. Similarly, males that invested heavily in mate assessment, but not relatively inactive males, preferred to affiliate with the relatively old, experienced female mating option. The ability to discriminate between naïve and reproductively competent mates thus appears dependent on particular characteristics of the selecting birds. However, the mechanism by which age and

Table 5.3 Variables affecting (a) female (n = 51) and (b) male (n = 36) affiliation behaviour in mate preference tests. Sexually experienced birds were offered a choice between a naïve and a competent mate. The dependent variable in each model was the percentage of active choice time spent affiliating with the competent bird. Models were qualitatively unaltered when mass was entered rather than condition, visual scores were entered rather than hue, non-preference-test song rate was used and the when the number of previous breeding attempts were entered rather than age (results not shown). (a) Female behaviour: test dyad identity: $Z = 1.11$, $P = 0.134$; chooser identity: $Z = 0.14$, $P = 0.443$; all interactions: $P > 0.305$. (b) Male behaviour: test dyad identity: $Z = 2.71$, $P = 0.007$; all other interactions: $P > 0.084$.

(a) Female mate preference

Variable	*df*	*F*	*P*	Parameter estimate (± SE)
Female chooser age	1, 35.3	4.54	**0.040**	1.97 (0.92)
Difference in song rate (test males)	1, 11.6	4.01	0.069	-0.22 (0.11)
Female chooser activity level	1, 40.9	0.23	0.631	0.19 (0.39)
Difference in body condition (test males)	1, 13.2	0.12	0.737	0.03 (0.09)
Difference in bill hue (test males)	1, 12.3	0.16	0.698	-0.01 (0.00)

(b) Male mate preference

Variable	*df*	*F*	*P*	Parameter estimate (± SE)
Male chooser age	1, 8.8	18.72	**0.002**	-1.67 (0.39)
Male chooser activity level	1, 31	5.68	**0.024**	0.54 (0.23)
Relative bill hue of test females (deviation from median)	1, 11.5	9.86	**0.009**	-0.18 (0.06)
Male chooser age by female bill hue interaction	1, 11.2	10.93	**0.007**	0.13 (0.04)
Difference in body condition (test females)	1, 6.2	0.24	0.642	-0.01 (0.02)

Figure 5.1 The degree to which females affiliated with competent males rather than naïve males increased with female age. For ease of presentation females are categorised here as having undertaken a single previous breeding attempt (n = 39 females aged between 25 and 27 months old) or two previous breeding attempts (n = 12 females aged 34-38 months old). Data were analysed with age (log-transformed) as a covariate and controlling for test dyad identity and chooser identity (see Methods). Relatively experienced, older females (one sample *t*-test: $t_{11} = 3.38$, $P = 0.006$), but not relatively inexperienced, younger females (one sample *t*-test: $t_{38} = 0.63$, $P = 0.536$) spent significantly more than 50% of their active choice time with reproductively competent males.

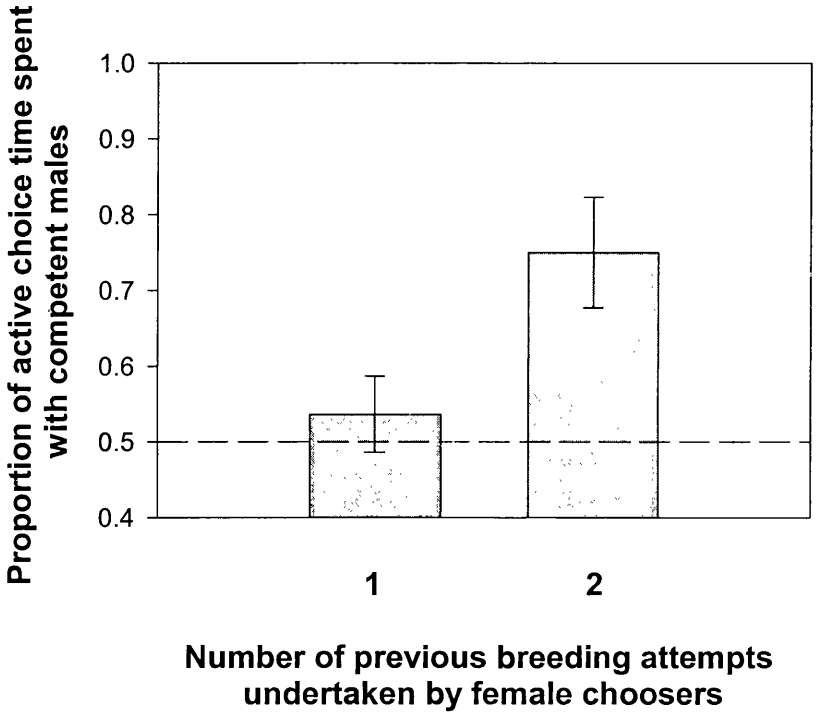


Figure 5.2 The degree to which males affiliated with competent females rather than naïve females increased with male activity level. Activity level was defined as the proportion of active choice time (see Methods) in which a male courted or faced towards either of the mating options. For ease of presentation males are categorised arbitrarily here as relatively active (n = 17 males with activity levels of > 35%) or relatively inactive (n = 19 males with activity levels < 35%). Data were analysed with activity level (log-transformed) as a covariate and controlling for test dyad identity (see table 3b). Relatively active males (one sample *t*-test: $t_{16} = 2.84$, $P = 0.012$), but not relatively inactive males (one sample *t*-test: $t_{18} = 0.39$, $P = 0.702$) spent significantly more than 50% of their time with reproductively competent females.

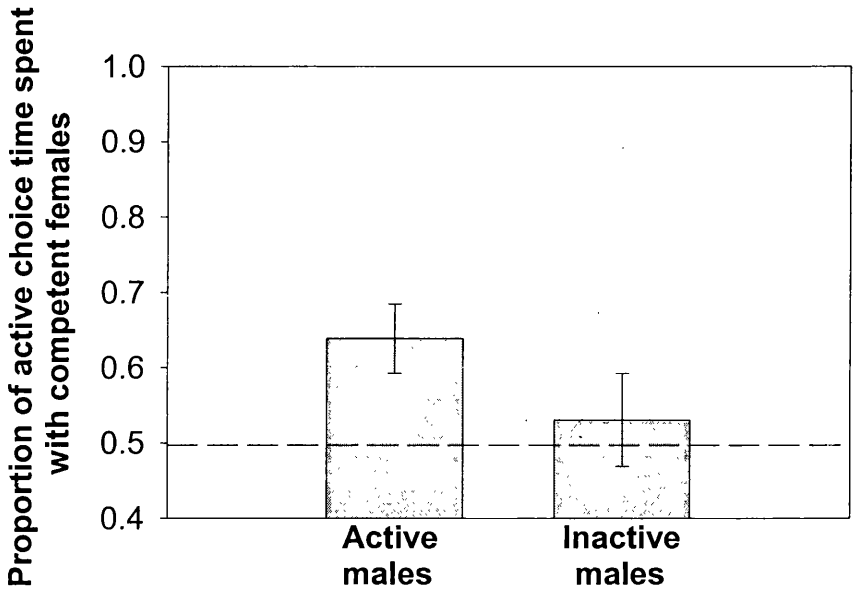
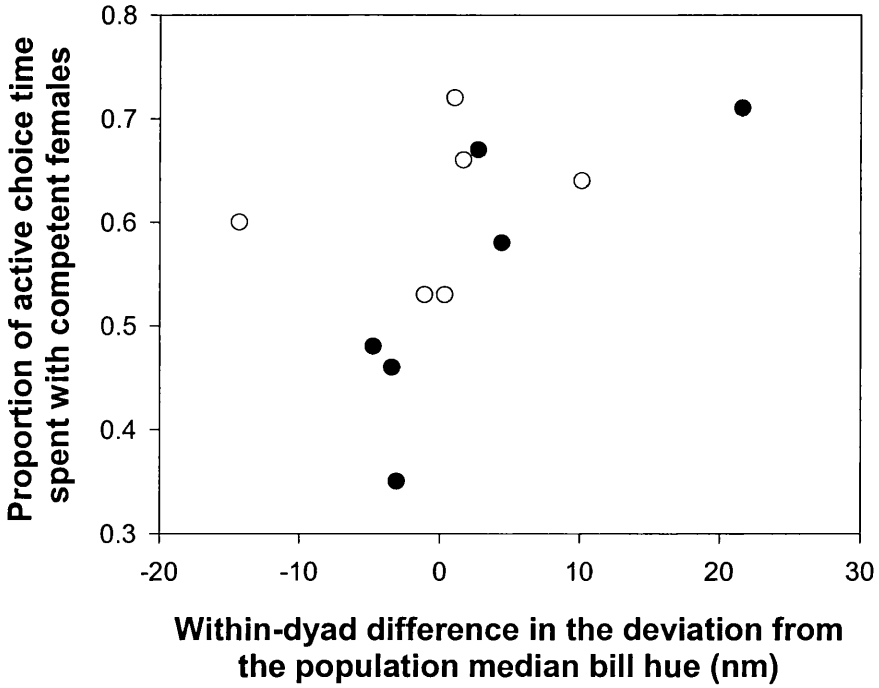


Figure 5.3 The relationship between male affiliation behaviour during preference tests, male age and the relative bill hue of test females. The analysis included male age and relative female bill hue as covariates and controlled for test dyad identity and male activity level (see table 5.3b). Here, each circle represents a test dyad comprising a competent female and a naïve female and male affiliation behaviour (see Methods) is averaged within each dyad. Each dyad was presented to three male choosers that were, on average, relatively old (closed circles; mean ages: 31 to 37.67 months) or relatively young (open circles; mean ages: 25 to 27 months). Within-dyad differences in the deviation of bill hue from the population median were calculated as the score of the naïve female minus the competent female. Thus positive values indicate that competent females have the more intermediate bill hues. Relatively old males (r = 0.75, n = 6, P = 0.089), but not relatively young males (r = 0.11, n = 6, P = 0.840) tended to affiliate more with the competent female when she possessed an intermediate bill colour in relation to the naïve female.



experience are signalled is not clear from the available data, as naïve and competent individuals did not differ in any measured trait.

No attempt was made to distinguish between age and breeding experience in this experiment. Both breeding experience and age independent of breeding

experience could affect the reproductive value of a mate. When birds become more efficient in parental care duties (e.g. incubation and brooding) with each successive breeding attempt, then it is adaptive to prefer experienced mates (reviewed in Forslund & Pärt 1995). Older birds can represent better mating options, regardless of breeding experience, when (1) general experience improves foraging efficiency and dominance rank (reviewed in Forslund & Pärt 1995); (2) old birds optimize reproductive effort towards the end of their reproductive life (Williams 1966); (3) reproductive performance and survival are positively correlated (Forslund & Pärt 1995); (4) mating with older individuals yields indirect genetic fitness benefits (the age indicator mechanism: Manning 1985; Kokko & Lindström 1996; Kokko 1998). The idea that older mates are genetically superior is predicated on the assumption that longevity is positively correlated with other fitness components (Kokko 1998). However, if trade-offs between early and late fitness traits exist, then this may favour the evolution of preferences for younger individuals (Hansen & Price 1995; Brooks & Kemp 2001). Moreover, direct fitness benefits should play a more important role than indirect fitness benefits in the mating decisions of species with biparental care (Reynolds & Gross 1990; Ryan 1997).

It is unclear what fitness benefits zebra finches gain by preferring older, more experienced mates. Williams & Christians (2003) examined the effects of age and experience on primary reproductive effort in the zebra finch and concluded that these factors are less important than nutritional condition in determining egg size and clutch size. They found that breeding experience did not influence primary reproductive effort, although older females on a low quality diet laid larger clutches and had shorter laying intervals. However, although primary reproductive effort does not appear to increase with experience, the effects of experience on incubation and rearing performance remain untested. A trend for an increase in fledging success with breeding experience was found in a wild Australian population (Zann 1994). It would be useful to investigate experimentally whether breeding experience influences incubation or rearing performance.

Schubert *et al.* (1989) found that older male zebra finches achieve greater pairing success than younger males in aviary colonies. They concluded that it was age *per se*, not previous breeding experience that elicited this mating skew. In contrast, Burley & Moran (1979) found that both male and female feral pigeons preferred mates with previous breeding experience rather than inexperienced mates, even when

the experienced mates were actually younger than the inexperienced mates. However, as a caveat they stress that there was, on average, only a 5-month age gap between experienced and inexperienced mates, therefore discrimination based on age may have been difficult. Further experiments are needed to tease apart effects of age and breeding experience on mate attractiveness. Nevertheless, in most natural situations, the two will be tightly coupled (Ens *et al.* 1996).

This study provided potential mates from opposite ends of the age continuum and the shape of the preference function for age among zebra finches is therefore unclear (Wagner 1998). In reality, there may be an optimal (intermediate) age of mate for zebra finches, resulting in discrimination against particularly young (and therefore inexperienced) birds and particularly old birds. Indeed, a preference for mates of intermediate age and experience has been found in studies of other species (Burley & Moran 1979; Jones *et al.* 2000). Even though they generally prefer experienced mates, feral pigeons actually prefer relatively young, inexperienced mates rather than very old (i.e. > 7 years), experienced mates (Burley & Moran 1979). The avoidance of very old mates is likely to be adaptive because reproductive performance will usually decline in old age as an expression of senescence (Comfort 1979; Forslund & Pärt 1995). The detrimental effects of senescence may offset any positive effects of previous experience. It is unclear at what age senescence begins to exert an effect in zebra finches, although birds of this species are sometimes capable of breeding at 6 years old (Immelmann 1965). My "old" birds were approximately 4 years old at the time of the tests and had bred within the previous 12 months. It may therefore be more appropriate to consider these birds to be of intermediate age if the entire life span of domesticated zebra finches (up to 7 years: Burley 1985) is taken into account (Jones *et al.* 2000).

Interestingly, I found that pair bond stability tended to be higher when males were paired with relatively young females with previous breeding experience, rather than relatively old (but experienced) females (chapters 3 & 4 – age range: 16 to 36 months). This suggests that when previous experience is comparatively uniform amongst a pool of potential mates, younger females are preferred because they have high reproductive potential. In species such as the zebra finch, in which pair bonds are often only severed by death (Zann 1996), the selection of a mate with high residual reproductive value is expected to be particularly important. The results of the current experiment suggest, however, that when older birds are also more experienced than

younger birds, male zebra finches base their mating decisions on experience not age. Conversely, females appear to prefer older males even when the prior breeding experience of mating options varies very little (chapters 3 & 4). Burley & Coopersmith's examination of zebra finch bill colour preferences (1987) also indicates that mate preferences should favour relatively young females and relatively old males, assuming that experience is held constant. Post-fledging mortality is higher in females than males (de Kogel 1997; also see chapter 7) and thus residual reproductive value declines more rapidly in females. This may provide a cogent explanation of the apparent sex-differences in age-related mate preferences in this species.

Both male and female preferences were influenced by their own previous experience. Females spent more time with competent males when they were themselves relatively old and experienced. Between-individual consistency in preferences amongst inexperienced feral pigeons is also low, whereas experienced birds generally prefer experienced mates (Burley & Moran 1979). Two explanations for experience-specific variation in preferences can be proposed: (1) birds may mate assortatively for quality (Burley 1977). However, this seems unlikely because males appear to prefer younger females when potential mates differ little in experience. In other words, young female choosers with previous breeding experience might be expected to be "high quality" mates. (2) Experience may allow birds to learn what represents a desirable mate and modify their preferences (for example, Collins 1995). Older male choosers did not spend more time than younger choosers affiliating with competent females, but the influence of female bill colour on male preference appeared to increase with male age. Males are expected to prefer females with bill colour in the middle of the phenotypic range (Burley & Coopersmith 1987) and these results indicate that there might be a learnt component to this preference. Males that spent a relatively large proportion of time observing and courting females (as opposed to merely being recorded near females) generally spent more time with experienced females than did relatively inactive males. Male activity is likely to correspond to assessment time, the duration of which should determine the accuracy of mate quality assessment (Sullivan 1994).

Competent and naïve birds did not differ in any of the phenotypic traits that I measured. However, following dyad formation, naïve males tended to have slightly redder bills than competent males within dyads. Nevertheless, no traits that I

measured influenced the preferences of either sex, except for the effect of female bill colour amongst older male choosers. Thus it is unclear which attributes of reproductively competent zebra finches cause them to be more attractive. Schubert *et al.* (1989) surmised that older, more experienced males may be preferred because their courtship displays are more polished as a result of their greater experience. The only aspect of courtship proficiency that I measured was male song rate and I found no differences between age classes. However, naïve and competent birds may have differed in other aspects of courtship that were not measured in this study, such as their co-ordination of courtship stages. Males and females are required to produce a complex series of courtship manoeuvres, in the right order and co-ordinated with a potential mate (Zann 1996). Alternatively, young birds may invest less in courtship than older birds (Proulx *et al.* 2002), although this seems unlikely, as zebra finches are relatively short-lived and appear to invest substantially in early-age life history parameters (Zann 1994; Birkhead *et al.* 1999).

Among socially monogamous animals, mates whose reproductive competence is established are generally expected to be preferred over naïve mates. The few studies that have examined these preferences have indeed found that relatively old, experienced mates enjoy higher mating success. However, it is important to consider that only certain individuals within a population may express such age-related preferences. Animals that have previously experienced a range of mates of varying quality, and animals that are relatively "choosy" and invest substantial effort in mate choice, may be most capable of discriminating between potential mates.

Chapter 6

Developmental nutrition and sexual attractiveness in zebra finches.

ABSTRACT

The quality of nutrition that an animal receives during ontogeny can affect its adult appearance. However, some animals, depending on the severity of nutritional deficit that they experience, are able to compensate and attain a normal adult appearance. Here I found that a dietary manipulation produced moderate effects on the nestling growth of zebra finches, *Taeniopygia guttata*, but these effects on body size and mass had disappeared by adulthood. In mate preference tests, I found that the proportion of time that males spent affiliating with females that had received a high quality (HQ) neonatal diet increased with the age of the male chooser and with his activity level. Females did not exhibit similar age- or activity-related preferences for HQ males, but generally preferred relatively heavy and red-billed males.

INTRODUCTION

The environment to which an animal is exposed during its development can have long-term effects on its adult appearance and attractiveness (Lindström 1999). Nutritional deficit, for example, can result in small adult size (Richner 1992) and reduced sexual signalling (Ohlsson *et al.* 2002). However, animals can often compensate for slowed growth once a nutritional restriction is lifted and subsequently achieve normal adult size and appearance (Arendt 1997). The extent to which growth compensation occurs will depend on the severity of the nutritional deficit, the importance of achieving a large size and the costs associated with accelerated growth (Arendt 1997; Metcalfe & Monaghan 2001), factors that may differ among species and between the sexes.

A useful species for studies of the effects of early nutritional environment is the zebra finch, *Taeniopygia guttata*. The extent to which adult appearance is permanently affected by developmental nutrition in this species depends on the nature, severity and timing of the early nutritional deficit. Nutritional conditions have been found to have permanent effects on adult mass and body size (Boag 1987; De Kogel 1997), on mass but not body size (Birkhead *et al.* 1999) and to have no effects on adult mass or size (Blount *et al.* 2003a). In some cases zebra finches thus appear to compensate for a nutritional deficit experienced during development. Moreover, females appear unable to distinguish between males that have received neonatal diets of different quality (Blount *et al.* 2003a). However, these recent studies have focussed principally on the how developmental nutrition affects male, rather than female, attractiveness (Birkhead *et al.* 1999; Blount *et al.* 2003a). Furthermore, these previous studies have not considered that mating preferences may depend on certain characteristics of the choosing birds, such as status and prior experiences, and can thus vary between individuals (Widemo & Sæther 1999).

In the present study, I examined the effect of neonatal diet quality on the growth of both male and female zebra finches. When these birds reached adulthood, I measured traits that are related to mate attractiveness, namely body mass (Wynn & Price 1993), bill colour (Burley & Coopersmith 1987), male song rate (Collins *et al.* 1994) and male song syntax (Clayton & Pröve 1989). In preference tests, sexually experienced males and females were offered a choice between test birds that had

received either a low-quality or high-quality neonatal diet. I examined whether the characteristics of the choosing birds determined their ability to discriminate between potential mates that had experienced different nutritional conditions during ontogeny.

METHODS

Neonatal diets

Pairs of zebra finches were randomly selected and allowed to breed under standardised conditions. Each pair was housed in a breeding cage (60 cm x 45 cm x 40 cm), at a room temperature of 21.25 ± 0.08°C and with access to an external nest-box. Prior to their chicks hatching, parents were fed a diet of *ad libitum* mixed seeds (foreign finch mixture; J. E. Haith, Cleethorpes, UK), cuttlefish, grit and water. In addition, each pair received approximately 1.5g of conditioning food (which comprised Rearing and Conditioning supplement (J. E. Haith), moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had been diluted 1g/litre with water) twice per week and 1.5g of green vegetables once per week.

Families were randomly assigned to receive one of three diets that began on the day that the first chick of a brood hatched, and ended when the youngest chick reached 17 days old. All three diets included *ad libitum* water and mixed seeds, including some that had been soaked overnight to saturation. The standard-quality (SQ) diet consisted of an additional 5g of conditioning food (see above) on two days per week (n = 11 pairs). The low-quality (LQ) diet included an additional 2.5g of conditioning food (see above) once weekly (n = 10 pairs). The high-quality (HQ) diet included an additional 5g of a mixture of conditioning food (see above) and homogenised hard-boiled egg on each day (n = 11 pairs). All birds received the same diet after day 17, which comprised *ad lib* mixed seed, water, grit and cuttlefish, and approximately 0.75g of conditioning food per bird once per week.

The diets differed in protein content, which was determined by the quantity and frequency with which the conditioning food and egg were provided. The mixed seeds contained 11.6% protein (percentage dry mass) and Haith's rearing and

conditioning supplement contained 13.6% protein (J. E. Haith, personal communication). Furthermore, egg was provided in only the HQ diet. Birkhead *et al.* (1999) calculated (after Etches 1996) that a diet consisting of *ad lib* seeds and 10g of hard-boiled egg per day comprises 42-46% protein. The HQ diet therefore provided more protein than the LQ and SQ diets. The HQ diet also provided higher concentrations of other nutrients found in the Daily Essentials 2 supplement (The Birdcare Company, personal communication; see Appendix).

Biometry

Body mass, tarsus length and wing length were measured when chicks were 10 and 17 days old. I measured birds on day 10 because this generally corresponds to the end of the linear growth phase of zebra finch chicks (Boag 1987). I measured birds on day 17 because this represented the end of the experimental treatment period and is the approximate fledging age of zebra finch nestlings (Zann 1996). Body mass was measured by using an electronic balance (± 0.1 g), and both tarsus length (± 0.1 mm) and wing length (± 0.1 mm) were measured initially using a graded glass slide with a wooden stop attached at one end. By day 17, and often by day 10, it was usually possible to measure tarsus length using a sliding calliper and wing length using a wing rule. Both tarsus and wing length were calculated from a mean average of three measurements. Body condition was calculated as the standardised residual of body mass regressed on tarsus and wing lengths. Wing length measurements of 6 birds and a single tarsus length measurement were not recorded on day 17. Body condition for these birds was calculated by regressing mass on tarsus and wing respectively. 13.5% of hatchlings (n = 89) died during the treatment period and only those birds surviving to day 17 were included in analyses of nestling biometry (n = 77; 26 LQ birds, 30 SQ birds, 21 HQ birds). All birds remained in their original family groups until independence at day 35, when they were transferred to unisex groups of 6 or 7 birds (cages of 120 cm x 45 cm x 40 cm). They remained in these groups until the mate preference tests. Pairs from the three diet groups did not differ in clutch size (LQ = 5.20 ± 0.25, SQ = 4.82 ± 0.33, HQ = 4.82 ± 0.35; $F_{2,29} = 0.47$, $P = 0.629$) or latency to lay (LQ = 8 ± 2 days, SQ = 10 ± 2 days, HQ = 8 ± 1 days; $F_{2,29} = 0.23$, $P = 0.794$).

I recorded the mass, tarsus length, wing length and bill colour of the birds once they reached adulthood (136.3 ± 1.4 days; n = 73, as 4 birds died in the first 3

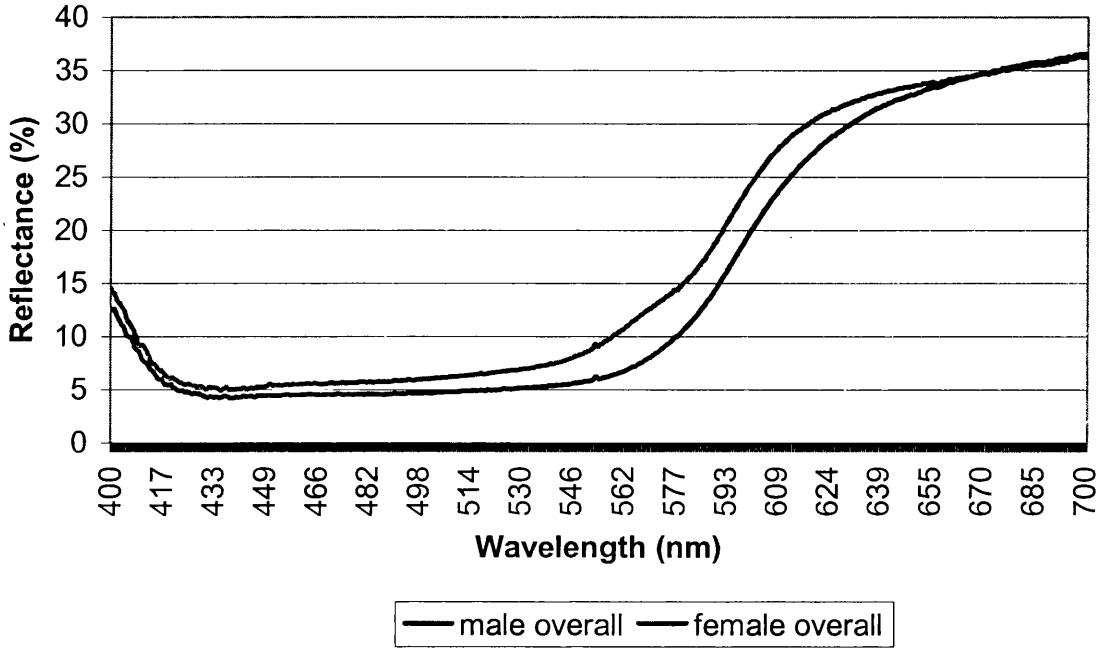
months after fledging, 25 LQ, 28 SQ, 20 HQ; the exact age at which measurements were made did not differ between treatments; two-way ANOVA: nestling diet, $F_{2, 67} = 0.53$, $P = 0.594$; sex, $F_{1, 67} = 0.57$, $P = 0.453$; nestling diet by sex interaction, $F_{2, 67} = 0.12$, $P = 0.892$). Bill colour was scored on a scale ranging from 1 (light orange) to 9 (dark red) using colour chips (Dulux Trade Colour Palette; Dulux, UK). These chips covered the full range of bill colours exhibited by the experimental birds (Blount *et al.* 2003a; see Appendix for full details of hue, chroma and brightness values).

I assessed bill colour by scoring again when birds were 246.2 ± 1.2 days old (n = 71, as two birds died following the initial measurements) and this time compared this measure to spectral analysis measurements. The reflected radiance of bills was measured in daylight conditions when birds were 254.3 ± 1.4 days old (n = 71) using a fibre optic spectrometer (S2000 Fiber Optic Spectrometer, Ocean Optics, Florida, USA). Birds were held under a tungsten-halogen lamp, with their mandible rested in a wooden notch. The spectrometer probe was then placed centrally on the maxilla at a 45° angle and reflectance recorded in a computer database. Five measurements per bird were recorded and mean values at 0.35nm intervals calculated. I calculated hue (as in Smiseth *et al.* 2001) as the wavelength corresponding to median reflectance $\left(\lambda\left[\frac{R\max + R\min}{2}\right]\right)$

Zebra finch bills reflect in the UV spectrum, as well as reflecting long wavelength light in the visible spectrum (figure 6.1). However, little is known about the precise role of UV signals in zebra finch mate preference formation (although see Bennett *et al.* 1996; Hunt *et al.* 2001). Therefore analyses focussed on long wavelength visible light emissions, which appear to be more important than UV signals in zebra finch mate choice (Hunt *et al.* 2001). Bill reflectance was measured on two separate dates by two different observers (n = 31 and 40 birds respectively). I controlled for measurement date in analyses of bill reflectance because this significantly affected hue (n = 71; influence of date on median reflectance: $F_{1, 68} = 105.83$, $P < 0.001$; sex: $F_{1, 68} = 46.56$, $P < 0.001$). Visual rankings of bill coloration were associated with hue calculated as median reflectance (males (n = 36): $F_{1, 33} = 29.51$, $P < 0.001$, controlling for measurement date: $F_{1, 33} = 81.44$, $P < 0.001$; females (n = 35): $F_{1, 32} = 6.00$, $P = 0.020$, controlling for measurement date: $F_{1, 32} = 84.86$, $P < 0.001$). This suggests that scoring assessment of bill colour closely approximates with an objective measurement

of bill hue. Hue calculated as λ_{slope} (as in Smiseth *et al.* 2001) gave qualitatively similar results (analysis not shown).

Figure 6.1 The mean reflected radiance of the bills of experimental birds (aged 246.2 ± 1.2 days old) over the human visible light spectrum (400-700 nm). Males (n = 36) and females (n = 35) are shown separately. Birds from different dietary treatments did not differ in their bill reflectance (table 2).



Mate preference tests

Ten birds of each sex were randomly chosen from both the HQ and LQ diet treatments to participate in mate preference tests. Birds of the same sex were paired into LQ-HQ test dyads. Three different birds were allowed to choose between members of each test dyad, but choosers were used in only a single test. As LQ and SQ birds did not differ in growth or adult traits (see statistical analysis section), I

selected only LQ birds for the tests. These tests were conducted when males were 305.3 ± 2.4 days old and females were 320.2 ± 2.7 days old. There was no difference in age between LQ and HQ birds entered into the mate preference tests (males: paired $t = 0.31$, $df = 9$, $P = 0.767$; females: paired $t = 0.95$, $df = 9$, $P = 0.368$). Sexually experienced birds from our stock population were offered a choice between a LQ mate and a HQ mate. These choosers were aged between 26 and 39 months old, had bred at least once before and had no prior contact with the birds from which they were allowed to choose. Tests were conducted under full spectrum artificial light (bird lamp, Arcadia, Croydon, UK).

Test dyads of LQ and HQ birds were matched for body mass, plumage colour and bill colour based on measurements on day 246.2 ± 1.2. Neither male body mass (paired $t = 0.27$, $df = 9$, $P = 0.794$) or female body mass (paired $t = 0.43$, $df = 9$, $P = 0.679$) differed between treatment groups at the time of the mate preference tests. Birds within test dyads had the same plumage colour (wild type grey, fawn or white). However, LQ males (colour score = 6.55 ± 0.30), at the time of the preference tests, had redder bills than the HQ males (5.85 ± 0.27) with whom they were paired (Wilcoxon signed ranks test: $Z = 1.98$, n = 10, $P = 0.048$). The bills of LQ females (2.90 ± 0.32) were slightly less red than bills of the HQ females (3.75 ± 0.36) with whom they were paired (Wilcoxon signed ranks test: $Z = 2.07$, n = 10, $P = 0.039$).

Experimental birds were placed in test cages (figure 3.1) and the preference tests were started after an acclimation period of 15 minutes. Preference tests followed an identical protocol to the one outlined in chapter 5. Each preference test lasted for 90 minutes. Male choosers spent 94.28 ± 1.14% (n = 30) of the total test time affiliating with the two female options and female choosers 90.72 ± 3.16% (n = 30). These proportions were labelled as the active choice times of the choosers (as in chapter 5). The proportion of active choice time spent with each dyad member was deemed to be a measure of mating preference. A dyad member was judged to be preferred as a mate when a chooser spent more than 50% of its active choice time with them.

Measurements of song performance

Song rate in preference tests was calculated using the same method that was detailed in chapter 5. I also measured song rate twice a few days after the preference tests

when males were 323.50 ± 2.23 and 340.50 ± 2.23 days old. LQ (n = 10) and HQ males (n = 10) were recorded (using an Aoi ECM-1025 microphone and Sony TCM-20DV recorder) singing to a previously unseen female for five minutes on both occasions. Males were placed alone in a cage, with the previously unseen female in an adjacent chamber. The mean proportion of time spent singing during both the first minute and the entire five-minute recording sessions were calculated. This was done by recording the number of phrases sung, and multiplying this by the mean phrase length (in seconds) to provide an estimate of the total amount of singing time. I also analysed song syntax using 28.65 ± 6.92 phrases per male (n = 20) from sonograms (using GW Instruments Soundscope package, GW Instruments, Inc., Massachusetts, USA). I recorded the number of different elements experimental birds had in their repertoires and the total number of elements contained in a full stereotyped phrase. These measures occasionally differed as some birds repeat elements within a phrase.

Statistical analysis

LQ and SQ birds did not differ in mass, tarsus length, wing length or condition on either day 10 or 17, or in adulthood (mixed models that compared the effects of the LQ and SQ diets, whilst controlling for brood identity as a random factor, brood size and sex: diet was non-significant in all models, $P > 0.568$). I therefore simplified all analyses of offspring traits by collapsing these two factor levels (Crawley 2002). Subsequent analyses compared HQ birds with both LQ and SQ birds pooled together (henceforth referred to as LQ birds). To check that collapsing the factor levels did not affect the fit of the models, I compared the "–2 Res Log Likelihood" (i.e. deviance) of the two-level diet and three-level diet mixed models, for all measurements (Crawley 2002). The difference in deviance, distributed as a χ^2 was significant for only 3 measurements, day-10 wing length, adult body mass (deviance was lower in the 3-level model in both cases) and adult condition (deviance was lower in the 2-level model). Treatment groups, however, did not significantly differ in these traits in either the 2- or 3-level models (i.e. the results were not qualitatively altered by using the 2-level models).

Analyses of population preferences were conducted using the mean averages from each dyad's set of three preference tests. However, male choosers varied in age (26 to 39 months old) and activity level (i.e. the proportion of active choice time spent

courting or facing one or other of the experimental females, a range of 0.15 to 0.63). Female choosers also varied in age (26 to 39 months old) and activity level (0.02 to 0.70). Mixed models were used to analyse if any traits of experimental birds and choosers explained a significant amount of the variation in mating preferences. The dependent variable in each model was the proportion of active choice time spent affiliating with the HQ bird. Within-dyad differences in traits were calculated as HQ minus LQ scores. Dyad identity was entered as a random factor. Entering body condition instead of mass produced qualitatively the same results (not shown). Males are expected to prefer intermediate female bill colour (Burley & Coopersmith 1987). I therefore analysed male mate preference for female bill colour using two different methods: (1) I assumed that redder bills would be preferred and I calculated within-dyad differences (HQ minus LQ score) in bill colour scores. (2) I assumed intermediate colours would be preferred; I calculated the median female bill index score (amongst all experimental females) and the deviation of each bird's bill index score from this median. Smaller deviations were expected to be more attractive to males. I calculated within dyad differences in deviation (LQ minus HQ). I ran separate models using both measures and their results were qualitatively similar. Chooser ages were log-transformed and proportional data were first arcsine square root transformed.

RESULTS

Brood size declined by 10.6% between hatching and sexual maturity (5.9% decline between hatching and fledging, and a further 5% decline between fledging and adulthood (day 136.3 ± 1.3)). However, the decrease in brood size did not differ between treatments (repeated-measures ANOVA: change in brood size over time, $F_{2, 36.7} = 6.34$, $P = 0.011$; change in brood size by nestling diet interaction, $F_{4, 36.7} = 1.28$, $P = 0.293$), nor did the average brood size during the nestling period ($F_{2, 29} = 0.81$, $P = 0.456$).

By day 10, birds that had received the HQ diet were significantly heavier and in better condition than LQ birds, but did not differ in tarsus length and wing length (table 6.1a). However, by day 17, which was the end of the diet treatment and the age

Table 6.1 Comparisons at (a) 10-days-old, (b) 17-days-old and (c) in adulthood of the biometry of zebra finches that received low- (LQ), standard- (SQ) or high-quality (HQ) neonatal nutrition. LQ & SQ birds did not differ in any measurement and are therefore pooled as LQ birds (see text). All analyses were mixed models, which controlled for brood identity as a random factor, sex, the interaction between sex and neonatal nutrition, and brood size. N = 56 LQ and 21 HQ birds ((a) & (b)); 53 LQ & 20 HQ birds (c). Brood size ($P > 0.104$) and the diet by sex interaction ($P > 0.132$) were always non-significant and sex ($P > 0.160$) was non-significant except for a few exceptions. Males (59.84 ± 0.25 mm) had longer wings than females (59.07 ± 0.33 mm) at day 17 ($F_{1, 52.4} = 8.06$, $P = 0.006$) and as adults (males = 59.92 ± 0.23 mm, females = 59.08 ± 0.33 mm, $F_{1, 54.7} = 8.64$, $P = 0.005$). Females (0.19 ± 0.15) were in better body condition than males (-0.18 ± 0.17) as adults ($F_{1, 65.4} = 4.49$, $P = 0.038$).

(a)

	Neonatal diet				
Day 10 trait	**LQ (±SE)**	**HQ (±SE)**	***df***	***F***	***P***
Body mass (g)	9.34 (0.28)	11.02 (0.36)	1, 28.9	4.19	**0.050**
Tarsus length (mm)	13.91 (0.26)	14.78 (0.30)	1, 28.5	1.46	0.236
Wing length (mm)	42.13 (1.10)	45.63 (1.30)	1, 28.0	2.39	0.133
Body condition	-0.21 (0.12)	0.56 (0.21)	1, 28.7	6.69	**0.015**

(b)

	Neonatal diet				
Day 17 trait	**LQ (±SE)**	**HQ (±SE)**	***df***	***F***	***P***
Body mass (g)	12.27 (0.28)	13.18 (0.33)	1, 30.2	0.80	0.378
Tarsus length (mm)	16.22 (0.20)	16.91 (0.14)	1, 24.7	1.19	0.285
Wing length (mm)	59.60 (0.22)	59.08 (0.49)	1, 20.5	1.93	0.179
Body condition	-0.12 (0.12)	0.10 (0.31)	1, 31.0	0.43	0.515

(c)

	Neonatal diet				
Adult trait	**LQ (±SE)**	**HQ (±SE)**	***df***	***F***	***P***
Body mass (g)	18.78 (0.32)	18.53 (0.59)	1, 29.6	0.14	0.715
Tarsus length (mm)	17.02 (0.10)	17.17 (0.17)	1, 29.3	0.28	0.603
Wing length (mm)	59.66 (0.21)	59.10 (0.49)	1, 24.1	2.10	0.160
Body condition	0.06 (0.14)	-0.15 (0.22)	1, 29.6	0.19	0.670

when birds were starting to fledge, there were no significant differences between treatments in body mass, body size, or body condition (table 6.1b). As adults, there remained no morphological differences between LQ and HQ birds (table 6.1c). There were also no differences in adult bill colour (table 6.2) and song characteristics (table 6.3) between birds from different treatment groups. In this sample of birds, body condition covaried only with bill colour ($r_s = 0.47$, n = 20, $P = 0.039$) but not with song rate calculated independently of preference tests ($r_s = 0.27$, n = 20, $P = 0.256$). Female body condition did not covary with their bill colour ($r_s = 0.26$, n = 20, $P = 0.264$).

In preference tests, male choosers overall showed no preference for HQ females (males affiliated more with the HQ female in 6 of the 10 dyads; two-tailed binomial test: $P = 0.754$). Males also did not court the HQ female more often, or direct more song towards her than they did with the LQ female (table 6.4a). However, older males (figure 6.2a) and more active males (figure 6.2b) spent more time with HQ females than did younger, less active males (table 6.5a). Male chooser age and activity level were unrelated ($r_s = 0.21$, n = 28, $P = 0.282$).

The number of dyads in which the HQ male was, on average, preferred (n = 5) was the same as the number in which the LQ male was preferred (n = 5). Females did not tend to spend more time with the HQ male or court more with him than they did with the LQ male (table 6.4b). However, females affiliated more with relatively heavy males and males with relatively red bills (table 6.5b). There was also a marginally non-significant tendency for females to affiliate more with males that possessed relatively complex song phrases (table 6.5b). Female preference did not vary with chooser age, but less active females spent more time with HQ males than did more active females (table 6.5b). This might be explained by the fact that the most active females generally preferred the males with the reddest bills (dependent variable: proportion of active choice time spent with the male with the reddest bill, n = 24 female choosers, in 2 dyads there was no bill colour difference; dyad identity (random factor): $Z = 1.28$, $P = 0.100$; female activity level: $F_{1, 22.0} = 6.14$, $P = 0.021$), which were generally LQ males (see Methods).

Table 6.2 Comparisons of the adult bill colours of birds that received either a low- (LQ), standard- (SQ) or high-quality (HQ) neonatal diet. Bill colour was scored on a scale of 1-9 when birds were 136.3 ± 1.4 (n = 53 LQ & 20 HQ birds) and 246.2 ± 1.2 (n = 51 LQ & 20 HQ birds) days old. Hue was calculated from the reflected radiance of bills (see Methods) when birds were 254.3 ± 1.4 days old (n = 71). Medians on a scale of 1-9 (first and third quartiles) are presented. Tests were two-way Kruskal-Wallis (Scheirer-Ray-Hare extension) ANOVAs (Sokal & Rohlf 1995) that controlled for sex. Analysis of hue also controlled for measurement date, as each bird was measured on one of two dates (see Methods). Sex was significant in all cases ($H_1 > 9.20$, $P < 0.002$). Males (5.50 (5.00, 6.50), n = 37) were visually assessed as possessing redder bills than females (3.50 (3.00, 4.00), n = 36) at approximately day 130. This remained the case by day 250 (males (n = 36): 7.00 (5.63, 7.00); females (n = 35): 5.00 (3.50, 7.00). Male bills had a longer wavelength of median reflectance (600.29 (595.73, 608.37)) than female bills (592.35 (586.59, 601.47)). All sex by diet interactions were non-significant ($H_1 < 1.04$, $P > 0.308$).

	Neonatal diet			
Bill colour trait	**LQ**	**HQ**	H_1	P
Bill score (day 130)	4.50 (3.50, 5.88)	4.13 (3.00, 5.50)	0.73	0.393
Bill score (day 250)	5.00 (3.50, 7.00)	5.00 (4.00, 6.38)	0.03	0.863
Reflectance hue (day 250) (nm)	597.76 (589.98, 604.84)	599.61 (591.75, 605.77)	0.05	0.823

Table 6.3 A comparison of the song performance of LQ and HQ males entered into mate preference tests. All analyses were paired *t*-tests (*df* = 9).

	Male neonatal nutrition			
Song performance measure	**LQ (±SE)**	**HQ (±SE)**	***t***	***P***
№ different elements in repertoire	4.70 (0.50)	5.20 (0.59)	0.76	0.464
Total № elements in full phrase	4.70 (0.50)	5.50 (0.58)	1.27	0.235
Song rate in tests (bouts min^{-1})	0.41 (0.11)	0.59 (0.15)	1.36	0.208
Proportion of time singing in non-preference test recording sessions (during initial 60 sec)	0.12 (0.04)	0.15 (0.05)	0.94	0.372
Proportion of time singing in non-preference test recording sessions (300 sec duration)	0.06 (0.02)	0.08 (0.02)	0.75	0.470

Table 6.4 The behaviour in mate preference tests of sexually experienced (a) males and (b) females. Choosing birds were given a choice between a sexually mature bird that had received low-quality (LQ) neonatal nutrition and one that had received high-quality (HQ) neonatal nutrition. Each test dyad was presented to three different choosers. All analyses were one-sample *t*-tests using LQ minus HQ values ($df = 9$).

(a)

	Female neonatal nutrition			
Male Behaviour	**LQ (±SE)**	**HQ (±SE)**	***t***	***P***
% time with female[a]	50.23 (6.91)	49.77 (6.91)	0.05	0.964
№ song bouts[b]	9.73 (2.95)	11.40 (3.50)	0.63	0.545
Song rate[c]	0.84 (0.27)	0.76 (0.19)	0.59	0.570
% total choice time courting[d]	16.27 (2.16)	19.21 (2.94)	0.57	0.582

(b)

	Male neonatal nutrition			
Female Behaviour	**LQ (±SE)**	**HQ (±SE)**	***t***	***P***
% time with male[a]	54.42 (8.66)	45.58 (8.66)	0.35	0.733
% total choice time courting[d]	18.90 (4.68)	10.24 (1.69)	1.12	0.291

[a] The percentage of active choice time (see Methods) that choosing birds spent with an opposite-sex bird.
[b] The total number of song bouts in a mate preference test.
[c] The number of song bouts per minute, during time spent the female.
[d] The percentage of active choice time during which the male was either facing toward the female or courting her.

Table 6.5 Variables affecting (a) male (n = 28, the ages of two male choosers were not known) and (b) female (n = 30) affiliation behaviour in mate preference tests. Sexually-experienced birds were offered a choice between mates that had been reared on either a low-quality (LQ) or high-quality (HQ) neonatal diet. (a) Male behaviour mixed model: test dyad identity: $Z = 1.50$, $P = 0.134$; all interactions: $P > 0.128$. (b) Female behaviour mixed model: test dyad identity: $Z = 1.63$, $P = 0.104$; all interactions: $P > 0.344$).

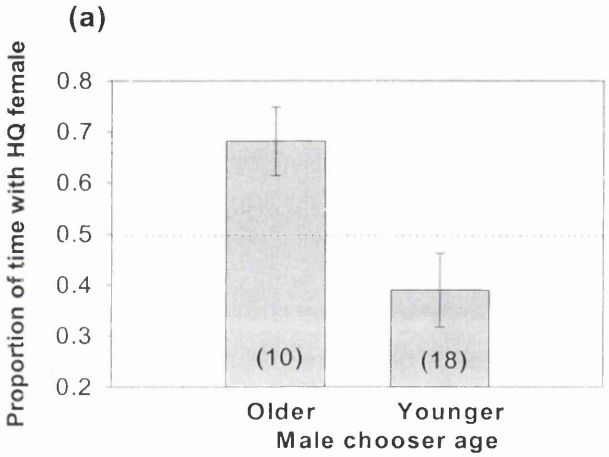
(a) Male mate preference for HQ female

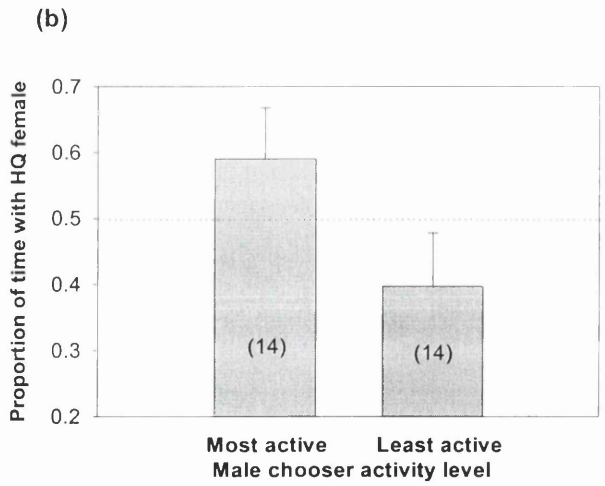
Variable	*df*	*F*	*P*	Parameter estimate (± SE)
Male chooser age	1, 22.2	13.98	**0.001**	2.21 (0.59)
Male chooser activity level	1, 21.9	5.42	**0.030**	0.51 (0.22)
Female difference in deviation from population median bill colour	1, 6.6	0.67	0.441	0.06 (0.07)
Female difference in body mass	1, 7.8	1.07	0.333	0.35 (0.34)

(b) Female mate preference for HQ male

Variable	*df*	*F*	*P*	Parameter estimate (± SE)
Female chooser activity level	1, 12.2	7.03	**0.021**	-0.70 (0.27)
Male difference in body mass	1, 7.5	23.35	**0.002**	2.51 (0.52)
Male difference in bill colour	1, 6.8	10.03	**0.017**	0.16 (0.05)
Male difference in song repertoire	1, 5.4	5.33	0.065	0.04 (0.02)
Male difference in song rate	1, 4.3	0.54	0.499	-0.07 (0.10)
Female chooser age	1, 13.5	1.19	0.294	-0.93 (0.85)

Figure 6.2 The extent to which males affiliated with females reared on a high-quality (HQ) diet (rather than females reared on a low-quality (LQ) diet) increased with (a) male age and (b) male activity level. For ease of presentation, males are grouped as (a) relatively young (26 to 28 months old, with a single previous breeding attempt) or relatively old (35 to 39 months old, with two previous breeding attempts); (b) relatively active (i.e. the 14 males that courted for the greatest proportion of their active choice time (see text)) or relatively inactive (i.e. the 14 least active males). Relatively old males (one-sample *t*-test: $t_9 = 2.70$, $P = 0.025$), but not relatively young males ($t_{17} = 1.54$, $P = 0.143$) spent more than 50% of their time with the HQ female. Neither the most active males ($t_{13} = 1.16$, $P = 0.265$) or the least active males ($t_{13} = 1.27$, $P = 0.226$) spent significantly more than 50% of their time with the HQ female.





DISCUSSION

While the quality of the neonatal nutritional environment to which experimental birds were exposed had some effects on their early growth patterns, birds from high-quality and low-quality nutritional backgrounds did not differ in any trait that I measured in them as adults. Furthermore, when all choosers were considered, it appeared that neither sex preferred mates that had been reared on a high-quality diet. Interestingly though, the amount of time that males spent affiliating with females that had received a high-quality neonatal diet varied and was dependent on particular characteristics of the choosing males.

The experimental diets produced only moderate effects on nestling growth. Nestlings receiving the high-quality diet were significantly heavier (by 17.99% on average) than other chicks by the end of their linear growth phase (i.e. the fastest period of nestling growth). By day 17, when birds were starting to fledge, this difference had disappeared and experimental birds remained apparently indistinguishable into early adulthood. Birds receiving relatively inferior nutrition were seemingly able to compensate for reduced neonatal growth even within the treatment period itself. The absence of adult morphological differences can be attributed to growth compensation, not differential mortality, because mortality did not differ between treatment groups. These results are consistent with previous studies of zebra finches in which birds recovered from restricted early growth. Birkhead *et al.* (1999), for example, employed a longer treatment period (day 0 to day 30) and used diets that were more divergent in quality than in this study. Even then, birds fed a low-quality diet compensated once the experimental diets ended, so that as adults they did not differ from HQ-diet birds in body size, bill colour or song rate. Growth compensation following an early nutritional deficit is a relatively common phenomenon among birds (Gebhardt-Heinrich & Richner 1998; Schew & Ricklefs 1998). Moreover, compensatory growth in terms of a number of adult traits is now well documented in the zebra finch (de Kogel 1997; Birkhead *et al.* 1999; Blount *et al.* 2003a; Spencer *et al.* 2003). Compensation may be especially important in short-lived species like the zebra finch, for which securing a mate early in life, and therefore looking attractive, is essential (Arendt 1997; Metcalfe & Monaghan 2001).

Recent studies have emphasised that mate preferences can vary between individuals when they possess different characteristics (Widemo & Sæther 1999; Basolo 2004; Forstmeier & Birkhead 2004). Choosers generally appeared unable to discriminate between potential mates from different nutritional backgrounds. However, both relatively old and relatively active males preferred females that had received a high-quality neonatal diet. This implies that even though experimental females were outwardly indistinguishable, males that are either relatively experienced (see Widemo & Sæther 1999) or invest substantial effort in mate assessment (Sullivan 1994) can discriminate between such females. Males that can recognise mates from high-quality nutritional backgrounds are likely to gain fitness benefits (Haywood & Perrins 1992). How these males discriminate is unclear. HQ females generally had slightly redder bills than LQ females at the time of the preference tests, but males are expected to favour females with intermediate bill colours (Burley & Coopersmith 1987). No evidence was found that males in this study preferred either the reddest bills or the most intermediate bill colours. Furthermore, female bill colour was also not associated with body condition. Males generally prefer females in good body condition (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001), but as females were matched for mass, this would have limited the variation in condition within test dyads.

Females did not discriminate between HQ and LQ males, regardless of their own age. However, females preferred relatively heavy males and males with relatively red bills. There was also an indication that females preferred males with relatively complex songs. These are male traits that have been established as female preference cues (Wynn & Price 1993; Burley & Coopersmith 1987; Clayton & Pröve 1989). It seems odd that the proportion of time that females spent with HQ males should decrease with female activity level. However, this may be a result of the female preference for red bills being dependent on female activity level. The most active females may have spent more time with LQ males because these males had slightly redder bills than their HQ dyad partners. These within-dyad differences may have occurred by chance because there were no differences in bill colour between treatment groups when birds were aged 250 days. It does, however, emphasise the importance of investment in secondary sexual traits for male zebra finches. Males that have experienced nutritional restriction early in life invariably compensate in terms of adult

bill colour, even when they fail to compensate in terms of adult morphology (de Kogel 1997) and antioxidant levels (Blount *et al* 2003a).

Developmental nutrition has previously been shown to affect male song complexity (Nowicki *et al.* 1998; Spencer *et al.* 2003). Zebra finches learn song from a single tutor, which is generally their father (Zann 1996). However, fathers were randomly allocated to treatment groups and would have varied in their song complexity. Therefore tutor song complexity was not controlled and this is liable to have masked any effect of early nutrition on offspring song learning in this experiment (see chapter 8 for a brother-brother comparison of song learning in different nutritional environments).

The nutritional environment experienced during development can have effects on appearance that persist into adulthood. However, animals for which securing a mate early in life is important may compensate for an early nutritional deficit when this is possible. This seems to be the case for zebra finches, although such compensation may carry costs (Birkhead *et al.* 1999; chapters 7 and 8). Moreover, even when compensatory growth occurs, birds that started life poorly may still appear less attractive as adults. Only the most experienced and choosiest individuals may be able to make such discriminations. In analyses of mate preference, it is important to consider the characteristics of both the individuals that are being selected and the individuals whose preferences are being measured.

APPENDIX

Dulux colour chip information

Chip number	Hue colour family	Light reflectance value	Chroma
1	68YR	34	780
2	55YR	28	778
3	44YR	26	756
4	33YR	20	708
5	31YR	18	648
6	15YR	16	594
7	19YR	13	558
8	09YR	11	476
9	14YR	10	434

Daily Essentials 2 content (after being diluted 1g/litre of water)

Vitamin A 2.4 $\mu g\ ml^{-1}$

Vitamin C 20.0 $\mu g\ ml^{-1}$

Vitamin D3 50.0 $\mu g\ ml^{-1}$

Vitamin E 0.025 $\mu g\ ml^{-1}$

Iodine 1.75 $\mu g\ ml^{-1}$

Iron 0.067 $\mu g\ ml^{-1}$

Folic acid 1.975 $\mu g\ ml^{-1}$

Also contains vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, biotin, choline bitartrate, niacin, pantothenic acid, selenium, cobalt, manganese, copper, magnesium, zinc, sulphur, sodium.

Chapter 7

Neonatal nutrition, growth and adult antioxidant capacity in zebra finches.

ABSTRACT

Animals from a range of taxa can recover from low-quality nutritional conditions experienced during development and attain normal adult appearance. This apparent recovery can, however, belie permanent adverse effects on a variety of traits related to survival. The quality of an animal's antioxidant defences is one such trait. In this study, I found that zebra finches, *Taeniopygia guttata*, that showed relatively slow neonatal growth, but had compensated by adulthood, also exhibited relatively low antioxidant capacity at 4 months of age. In a separate experiment, birds that experienced a neonatal nutritional restriction, but on this occasion did not compensate once it was lifted, had relatively high antioxidant capacity at 17 months of age. I discuss how these results may be interpreted in light of previous studies of developmental nutrition and antioxidant defences.

INTRODUCTION

The conditions experienced during early development can have profound and persistent effects on the adult phenotypes of animals (Lindström 1999; Metcalfe & Monaghan 2001; West-Eberhard 2003). In particular the quality of nutrition received early in life can influence traits related to mating success (Nowicki *et al.* 1998; Ohlsson *et al.* 2002; Buchanan *et al.* 2003) and longevity (Desai & Hales 1997; Aihie Sayer *et al.* 2001; Aihie Sayer & Cooper 2002), both of which are components of lifetime reproductive success. Sometimes, however, individuals are able to achieve normal adult size and appearance despite a nutritional deficit during ontogeny (Arendt 1997). Yet an ostensibly normal phenotype can mask underlying long-term problems resulting from the poor early nutritional environment (Metcalfe & Monaghan 2001). For example, even when individuals recover from a period of retarded growth by compensating in terms of body size and secondary sexual traits, they may still exhibit reduced life spans (Lucas *et al.* 1996; Birkhead *et al.* 1999). It has therefore been suggested that compensatory growth may in itself carry long-term costs (Metcalfe & Monaghan 2001; Metcalfe & Monaghan 2003).

A poor nutritional environment during development may cause a permanent reduction in an organism's capacity for self-maintenance, such as the ability to prevent oxidative damage. Oxidative damage is caused by free radicals and other reactive molecules produced during oxygen reduction (Beckman & Ames 1998). Organisms can either prevent such damage by employing antioxidant compounds and scavenging enzymes (primary defences) or they can utilise an array of intracellular enzymes to repair damage (secondary defences) (Yu 1994). When levels of reactive oxygen species exceed an organism's capacity to defend against them, this can result in oxidative stress, which is liable to damage cells. Oxidative stress is consequently believed to be a key determinant of the rate at which organisms age (Beckman & Ames 1998). For example, transgenic strains of *Drosophila melanogaster* that exhibit increased levels of antioxidant defence have significantly extended life spans (Fleming *et al.* 1992).

Zebra finches, *Taeniopygia guttata*, can show varying degrees of compensatory growth following an early nutritional deficit (Birkhead *et al.* 1999; Blount *et al.* 2003; Spencer *et al.* 2003). However, birds that attain normal adult

appearance after experiencing poor early developmental conditions may still have a reduced life span (Birkhead *et al.* 1999). Previously it has been shown that a brief (15 days post-hatching) period of low-quality neonatal diet reduces adult plasma concentrations of the lipophilic antioxidants vitamin A, total carotenoids and vitamin E (tocopherol isomers) in this species (Blount *et al.* 2003). These results suggest that long-term survival may be reduced by developmental stress by permanently impairing the uptake of dietary lipophilic antioxidants. However, lipophilic antioxidants form only part of a battery of antioxidant defences. Other important antioxidant scavenging components include plasma lipophilic molecules such as bilirubin and a variety of flavonoids, hydrophilic antioxidants such as vitamin C (ascorbic acid), uric acid (Stinefelt *et al.* 2005), cysteine, certain flavonoids and reduced glutathione, and cellular (enzymatic) systems (reviewed in Yu 1994). Using a more general measure of an animal's antioxidant capacity should provide a more accurate indication of its ability to resist oxidative stress than do measurements of specific antioxidant components.

My aim in this study was to examine whether low-quality neonatal nutrition impairs the plasma antioxidant capacity of adult zebra finches. The long-term implications of neonatal nutritional stress may vary over the course of an animal's life and with the severity of the nutritional deficit; therefore I conducted two separate experiments that differed in the ages and nutritional backgrounds of the experimental birds. In both experiments I manipulated diet quality amongst nestlings. The divergence in diet quality between the treatment groups was greater in experiment 2 than it was in experiment 1. In experiment 1, I measured the antioxidant capacity of both males and females from different dietary backgrounds when birds were approximately 130 days old. In experiment 2, I again measured antioxidant capacity, but this time when birds were approximately 500 days old.

METHODS

EXPERIMENT 1

Neonatal diets, biometry and blood sampling

Experimental birds were selected from the low-quality (LQ), standard-quality (SQ) and high-quality (HQ) dietary groups detailed in chapter 6. Thus the neonatal diets and biometry protocol follow those outlined in that chapter. Five or six unrelated birds of each sex from each of the three treatment groups were selected to be blood sampled. A single bird was selected from each of the 32 broods to ensure that sampled birds were unrelated. Birds were randomly selected from within each brood, whilst ensuring that an even number of males and females were chosen. Blood samples (up to 140 μl) were taken under UK Home Office licence when birds were adults (130.6 ± 1.7 days (n = 32); the exact age at which samples were obtained did not differ between treatments; two-way ANOVA: nestling diet, $F_{2,26} = 0.01$, $P = 0.994$; sex, $F_{1,26} = 0.06$, $P = 0.804$; nestling diet by sex interaction, $F_{2,26} = 1.89$, $P = 0.171$). Standard sampling procedure, which suggests that in birds it is safe to withdraw up to 10% of total blood volume (Campbell 1995), was followed. Blood was taken from the brachial vein, centrifuged and plasma was stored at –20 °C prior to antioxidant analysis. I obtained 29 (LQ = 10, SQ = 9, HQ = 10) useable (i.e. non-cloudy) blood plasma samples from the 32 individuals.

EXPERIMENT 2

Neonatal diets

Zebra finches with previous breeding experience were randomly paired and allowed to breed in individual cages (60 cm x 45 cm x 40 cm). Pairs were kept on a 12:12 light dark cycle, at a room temperature of 21.25 ± 0.08°C and fed on a standard diet. The standard diet consisted of *ad lib* mixed seed (foreign finch mixture, J. E. Haith, Cleethorpes, UK) and water, plus, three times per week, 5g of rearing and conditioning supplement (J. E. Haith, moistened at a 3:2 mass ratio with Daily

Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had been diluted 1g/litre with water). When the first chick in each nest hatched, pairs were allocated randomly to one of two dietary treatment groups. The low-quality (LQ) diet consisted only of *ad lib* mixed seed and water, but no further dietary supplements. The LQ and SQ diets in experiment 1, which included some supplements (see chapter 6), were both superior to the LQ diet used in this experiment. The high-quality (HQ) diet included additional cuttlefish, and 10g of a mixture of Haith's supplement and homogenised egg every day, which was first soaked in 15ml of Daily Essentials 2 supplement (dilution of 1g/litre) (see chapter 6 for details of the nutrient content of each dietary component). Families on the HQ diet in experiment 2 thus received twice the quantity of conditioning food and egg than the HQ families in experiment 1. I fostered siblings on the day of hatching to different families, receiving either the LQ or a HQ diet. The difference between the LQ and HQ diets were more extreme in this experiment than in experiment 1. After fostering each brood consisted of 3 unrelated chicks. Foster fathers were removed when the oldest chick reached 15 days as part of a song-learning experiment (see chapter 8). Each brood of 3 chicks was thereafter raised to independence by its foster mother. The experimental diet lasted for the 20-day nestling period, after which all fledglings received the same standard diet (see above). Offspring were removed from their foster mother when they reached 35 days and were transferred to single sex groups of 4-5 individuals.

Biometry and blood sampling

I compared siblings of the same sex that received opposite diet treatments (n = 17 sibling pairs; 7 brother-brother pairs and 10 sister-sister pairs). Using analyses with full siblings is advantageous as this partially controls for genetic factors that may influence growth and antioxidant capacity. I measured mass (± 0.1g) on the day of hatching, prior to chicks being fostered and on every second subsequent day until the end of the diet treatment period on day 20. I measured the body mass of adults on day 70, when birds had reached their fully-grown size (Boag 1987) and on day 120. I measured tarsus length (± 0.1mm) and wing length (± 0.5mm) on day 120, and head-bill length (± 0.1mm) and bill length (± 0.1mm) on day 150. Bill length was measured from the tip of the bill to the anterior edge of the nostrils because this produces less measurement error than measurements of total culmen (Borras *et al.* 1999). The four

measures of adult body size were entered into a factor analysis to produce a single body size score for each bird (1st principal component: eigenvalue of 2.38, explaining 59.5 % of total variance). Unlike in experiment 1, I was able to calculate growth rates because I measured all experimental birds at set ages throughout their life. Mass increment for the diet treatment period (day 0 to 20) and the post-treatment period (day 20 to 70) were calculated (instantaneous growth rates were calculated as log $mass_2$ – log $mass_1/time_2$ – $time_1$). The zebra finch is a size-monomorphic species (Zann 1996) and there were no sex differences in growth rates either during treatment (*t*-test: $t_{32} = 0.81$, $P = 0.424$) or post-treatment (day 20 to 70) ($t_{32} = 1.29$, $P = 0.206$). Blood samples were taken from sibling pairs when birds were aged 510 ± 3 days (n = 26) using the same protocol as experiment 1. In 13 of the 17 sibling pairs both sibs were still alive and blood was taken from these 26 birds. Of the four deaths prior to sampling, 2 were LQ females, 1 was a LQ male and 1 was a HQ female.

PLASMA ANTIOXIDANT ACTIVITY ASSAY

The $ABTS^+$ decolourisation assay (Re *et al.* 1999) was used to determine the Trolox equivalent antioxidant capacity (TEAC) (sometimes referred to as antioxidant activity, see Huang *et al.* 2005) of the blood plasma of the experimental birds. This method is based on the capacity of plasma antioxidants to scavenge the $ABTS^+$ radical cation, relative to Trolox (a vitamin E analogue with known antioxidant activity). An $ABTS^+$ stock solution was prepared by dissolving 0.0192g $ABTS^+$ in 5ml dH_2O and adding 88μl of potassium persulphate solution (0.1892g $K_2S_2O_8$ in 5ml dH_2O). This stock solution was vortexed briefly and incubated in darkness at room temperature overnight to allow the stabilisation of the radical. Trolox solutions were made by dissolving 0.031286g Trolox in 25ml 100% ethanol, then adding 100μl of this solution to 900, 400, 275 and 150μl of phosphate buffered saline (PBS). Defrosted plasma was diluted 1:4 with PBS and centrifuged at 13,000rpm for 30 minutes at 0°C. The lower phase was then decanted and spun again at 13,000rpm for 5 minutes at 0°C. 1ml diluted $ABTS^+$ solution (1ml $ABTS^+$ stock solution with approximately 88ml PBS solution) was pipetted into a glass cuvette and its absorbance reading measured in a spectrophotometer. 10μl PBS was then pipetted into the cuvette and a timer started. The $ABTS^+$ and PBS were mixed gently and placed back into the spectrophotometer. When the timer reached 1 minute the

spectrophotometer was activated to scan for 1 minute. The change in absorbance over the two-minute period thus represented the baseline reading for a solution without antioxidant activity. This procedure was repeated with each of the 4 Trolox dilutions and all the plasma samples. Trolox equivalent values, representing the concentration of Trolox required to produce an equivalent antioxidant potential, were calculated for each plasma sample with reference to a Trolox standard calibration curve.

STATISTICAL ANALYSIS

I tested models in which TEAC was the dependent variable and sex and diet were fixed factors. The experiment 2 models contained sibship as a random factor, which remained in all final models. Body size and mass at day 10, fledging and adulthood, as well as estimates of growth rates were entered as covariates. For experiment 1, I ran these models with diet as either a 2- or 3-level factor (i.e. with LQ and SQ diet levels separate or collapsed; see chapter 6) and the model results did not differ. Variables that were closely associated, and were thus confounded (such as diet and day 10 mass in experiment 1 and diet and growth rates in experiment 2) were not used in the same model. I used backward elimination of non-significant effects, starting with non-significant interactions. In experiment 1 models, adult body mass and the percentage of adult tarsus length gained between day 10 and adulthood were log-transformed prior to analyses. In experiment 2 models, growth rate between day 20 and 70 was log-transformed prior to analysis.

Logistic regression analysis was used to examine whether either TEAC or early growth affected the survival of experimental birds from experiment 1. Variables were entered using a stepwise method when $P < 0.05$ and removed using the likelihood ratio criterion. The dependent variable was status (alive or dead) at day 750.

RESULTS

EXPERIMENT 1

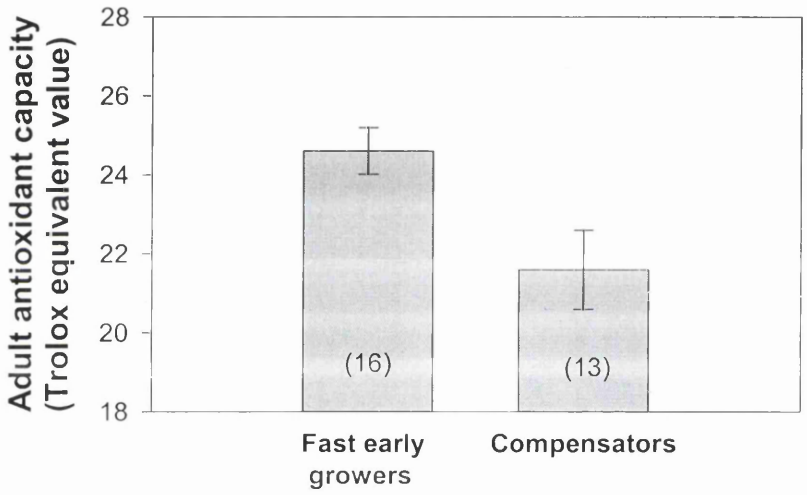
The effects of the LQ, SQ and HQ neonatal diets on growth and adult biometry are detailed in chapter 6. Briefly, LQ and SQ offspring did not differ in any body measurement during development or as adults. HQ offspring were heavier than other nestlings on day 10, but as adults they were the same size and weight as LQ and SQ birds, having apparently compensated for the early nutritional deficit.

Table 7.1 Adult antioxidant capacity in experiment 1 was explained by four single-parameter models. N = 29. Neonatal diet, sex and all interaction terms were non-significant in all models (see text).

Variable	$F_{1, 27}$	P	Effect estimates
Day 10 mass (g)	7.12	**0.013**	+
Day 10 tarsus length	4.32	**0.047**	+
% of adult mass gained between d10 & adulthood	5.38	**0.028**	-
% of adult tarsus gained between d10 & adulthood	5.40	**0.028**	-

TEAC of these birds when aged 130 days old did not differ between diet treatments (LQ (n = 10) = 24.04 ± 0.84; SQ (n = 9) = 21.48 ± 1.52; HQ (n = 10) = 24.07 ± 0.61; diet was non-significant in all of the tested models: $F_{2, 26} < 2.02$, $P > 0.154$). Males and females did not differ in TEAC ($F_{1, 24} < 0.50$, $P > 0.486$ in all models) and all interaction terms were non-significant ($P > 0.139$ in all models). There were four single-parameter models with significant effects (table 7.1). However, in a stepwise multiple regression, only day 10 mass predicted adult TEAC (day 10 mass: $t = 2.67$, $P = 0.013$; day 10 tarsus length: $t = -0.79$, $P = 0.438$; % of adult tarsus length gained between day 10 and adulthood: $t = -0.64$, $P = 0.644$; % of adult mass gained between day 10 and adulthood: $t = -0.20$, $P = 0.841$). Relatively light 10-day-old nestlings subsequently had relatively low TEAC as adults. These birds tended to compensate by gaining a greater proportion of adult mass and body size than heavy, large nestlings between day 10 and the time of blood sampling (see

Figure 7.1 The plasma antioxidant capacity of birds aged 130.6 ± 1.7 days in relation to their growth pattern. Day-10 mass and the percentage of adult mass gained between day-10 and adulthood were negatively correlated (see Methods) and were entered into a factor analysis. This produced a single "growth pattern" score for each bird (eigenvalue = 1.86; 93.10% of variance explained). Birds either grew relatively fast as neonates and relatively slowly thereafter (i.e. positive factor scores, labelled "fast early growers", which included 8 HQ, 3 SQ & 5 LQ birds) or grew relatively slowly up to day 10 and relatively quickly thereafter (i.e. negative factor scores, labelled "compensators", which comprised 2 HQ, 6 SQ & 5 LQ birds). Antioxidant capacity was significantly different between these two groups (t_{27} = 2.69, P = 0.012). Brackets show the total numbers in each group.



chapter 6 results). Offspring that grew relatively fast as neonates and relatively slowly thereafter had higher TEAC than birds that showed the opposite pattern by growing relatively slowly to day 10 and relatively quickly thereafter (figure 7.1). Reduced nestling growth and fast (compensatory) growth after day 10 were closely associated and their relative importance in producing low adult TEAC is therefore unclear. There was no association between TEAC and adult bill colour (r_s = -0.14, n = 29, P = 0.458; males: r_s = -0.22, n = 16, P = 0.408; females: r_s = 0.07, n = 13, P = 0.834). This was the case separately among HQ birds (males: r_s = 0.32, n = 5, P = 0.604; females: r_s = 0.71, n = 5, P = 0.182) and experimental birds from the other two diet groups (males: r_s = -0.42, n = 11, P = 0.196; females: r_s = -0.07, n = 8, P = 0.862).

Females from the three neonatal diet treatments did not exhibit different survival rates between day 17 and 750 (Kaplan-Meier survival analysis: LQ mean survival time = 671 ± 53 days; SQ = 601 ± 54 days; HQ = 689 ± 27 days; log rank = 1.54, $df = 2$, $P = 0.463$). Similarly, the survivorship of males did not differ between treatment groups (LQ = 704 ± 45 days; SQ = 657 ± 54 days; HQ = 616 ± 70 days; log rank = 1.75, $df = 2$, $P = 0.417$). These results were not altered qualitatively by collapsing LQ and SQ factor levels (as performed in chapter 6; results not shown). Neither TEAC at approximately 130 days or day 10 mass predicted survival (logistic regression: all $df = 1$; variable in equation: sex: Wald = 4.74, $P = 0.029$; variables not in equation: TEAC: score = 0.74, $P = 0.389$; day 10 mass: score = 1.90, $P = 0.168$; TEAC x sex: score = 0.16, $P = 0.694$; day 10 mass x sex: score < 0.01, $P = 0.954$). Males had higher survival than females (93.75% v 53.85% of blood-sampled males and females, respectively, survived to day 750; 82.50% v 64.86% of all fledgling males and females survived to day 750).

EXPERIMENT 2

LQ birds grew slower during the treatment period (day 0 to 20) than their HQ siblings and were significantly lighter than their HQ sibs by day 20 (table 7.2). LQ birds, however, grew faster between the cessation of diet treatments and day 70, by which age they had reached their adult size (table 7.2). Thus as adults there were no significant differences in mass between LQ and HQ sibs, although permanent differences in body size remained, which was not the case in experiment 1 (table 7.2). However, there was considerable variation in the extent to which LQ birds grew slower during treatment and faster after treatment than their HQ sibling (coefficient of variation (CV) of sibling difference (LQ minus HQ) in day 0 to 20 growth rate = 75.5%; CV of sib difference in day 20 to 70 growth rate = 90.6%). Furthermore, the LQ birds that grew slowest in comparison to their HQ sibling prior to day 20 were not necessarily the same LQ birds that showed the most extreme compensatory responses (correlation between sibling differences in day 0 to 20 growth rate and day 20 to 70 growth rate: $r = -0.306$, n = 17, $P = 0.233$). Thus, unlike in experiment 1, early growth deficit amongst LQ birds and compensatory responses were not associated.

Although the LQ and HQ diets in experiment 2 were more divergent than those in experiment 1, there were no differences between experiments in the day 10

mass of LQ offspring (experiment 1: 9.3 ± 0.3g, n = 56 (LQ and SQ combined); experiment 2: 9.3 ± 0.4g, n = 17; $t_{71} = 0.01$, $P = 0.995$) or HQ offspring (experiment 1: 11.0 ± 0.4g, n = 21; experiment 2: 11.1 ± 0.4g, n = 17; $t_{36} = 0.24$, $P = 0.813$). HQ offspring did not differ in adult body mass (experiment 1 (approximately day 130): 18.5 ± 0.6g, n = 20; experiment 2 (day 120): 18.0 ± 0.7g, n = 17; $t_{35} = 0.55$, $P = 0.586$). However, LQ birds from experiment 1 were generally heavier as adults than LQ birds from experiment 2 (experiment 1 (approximately day 130): 18.8 ± 0.3g, n = 53; experiment 2 (day 120): 17.4 ± 0.7g, n = 17; $t_{68} = 2.06$, $P = 0.043$). Thus the extent to which LQ birds compensated was generally greater in experiment 1.

Table 7.2 Paired comparisons of the growth and morphology of same-sex siblings in experiment 2 that received LQ and HQ diets (see Methods) respectively for 20 days post-hatching. All tests are paired t-tests conducted on full-sibling pairs. These birds were raised by foster parents until day 15 and their foster mother thereafter until day 35. All foster broods comprised 3 nestlings. N = 17 sibling pairs (7 brother-brother pairs and 10 sister-sister pairs). $Df = 16$.

	Neonatal diet			
Measurement	**LQ sib (±SE)**	**HQ sib (±SE)**	***t***	***P***
Body mass at day 0 (g)	1.0 (0.0)	1.0 (0.0)	1.16	0.262
Growth rate day 0 to 20	5.3×10^{-2} (9.0×10^{-4})	5.6×10^{-2} (8.5×10^{-4})	2.70	**0.016**
Body mass at day 20 (g)	11.6 (0.4)	13.4 (0.3)	5.61	**< 0.001**
Growth rate day 20 to 70	3.3×10^{-3} (0.3×10^{-3})	2.2×10^{-3} (0.2×10^{-3})	3.07	**0.007**
Body mass at day 70 (g)	16.8 (0.5)	17.4 (0.5)	1.23	0.237

LQ birds did not differ from their HQ siblings in their TEAC at approximately 510 days old (Trolox equivalent values of LQ birds = 27.4 ± 0.8; HQ birds = 25.9 ± 0.7; paired *t*-test: $t_{12} = 1.39$, $P = 0.191$). However, the interaction between diet and mass at day 70 was significant (table 7.3). Amongst HQ birds, TEAC was not

associated with day 70 mass, but LQ birds that reached a relatively low mass by day 70 (i.e. compensated little for their early nutritional deficit) had relatively high TEAC (figure 7.2). Overall birds from this experiment had higher antioxidant capacities when aged 510 ± 3 days (mean Trolox equivalent value of 26.66 ± 0.55, n = 26) than birds from experiment 1 that were aged 131 ± 2 days (23.26 ± 0.61, n = 29) (t_{53} = 4.08, $P < 0.001$).

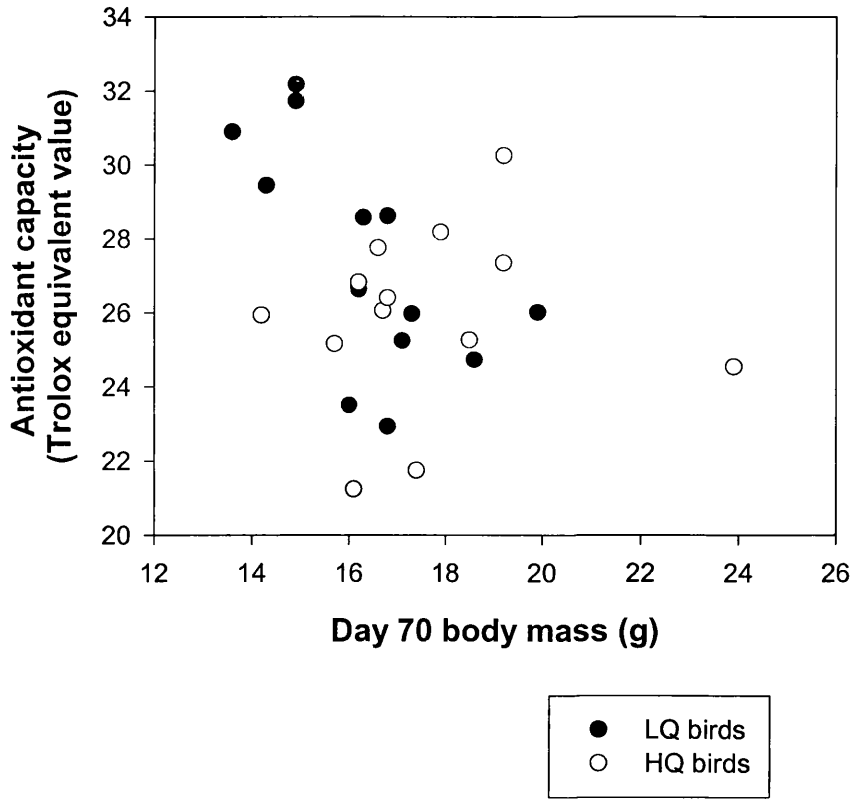
Table 7.3 Variables affecting plasma antioxidant capacity of zebra finches from experiment 2 aged approximately 510 days old. Original (biological) family did not influence antioxidant capacity ($Z = 0.11$, $P = 0.455$). All other interactions were non-significant ($P > 0.496$).

Variable	*F*	*df*	*P*
Diet	6.41	1, 12.4	**0.026**
Day 70 mass	4.20	1, 17.8	0.055
Diet by day 70 mass interaction	5.85	1, 12.5	**0.032**
Sex	0.37	1, 11.8	0.552

DISCUSSION

The influence of growth and development on the antioxidant defences of adult zebra finches differed between the two experiments. In experiment 1, the antioxidant capacity of birds aged approximately 4 months old was strongly correlated with both body mass and size when individuals were 10 days old. Hatchlings that initially grew relatively slowly had low antioxidant capacity in early adulthood. These results suggest that stressful conditions during early development can reduce antioxidant defence capabilities in later life. However, in a second experiment, where LQ birds experienced a greater level of nutritional stress during early development, no direct association was found between nestling growth and antioxidant capacity in birds aged approximately 17 months. In this experiment, the receipt of a low-quality neonatal diet generally reduced nestling growth in relation to siblings that received a high-

Figure 7.2 The effect of neonatal diet and adult body mass on the antioxidant capacity of zebra finches aged approximately 510 days old. Birds received either a low-quality (LQ) or a high-quality (HQ) diet for the first 20 days after hatching (see Methods for diet details). Amongst HQ birds, body mass at day 70 and antioxidant capacity were not associated (r_{11} = 0.09, P = 0.772). However, LQ birds that achieved a relatively high mass by day 70 had lower antioxidant capacity than those LQ birds that remained light as adults (r_{11} = -0.65, P = 0.017). There were no sex differences in antioxidant capacity. N = 26. Analysis was performed on 13 pairs of same-sex siblings that had received different neonatal diets, with sibship entered as a random factor.



quality diet. Some LQ siblings compensated for this reduced growth after the dietary restriction ended, but some remained relatively small into adulthood. The highest

antioxidant capacities in adulthood were found amongst these LQ birds that remained relatively light.

The results of experiment 1, in tandem with previous work in zebra finches (Blount *et al.* 2003), suggest that adult antioxidant defence capabilities may be determined at a very early stage in life. Nutritional programming is known to permanently change the quantity and functioning of specific cell types (Desai & Hales 1997; Lucas 1998). The assay employed in this experiment measured the reducing capacity of plasma antioxidants that function by electron transfer (Huang *et al.* 2005), which include not only vitamin A, carotenoids and vitamin E, but also flavonoids, vitamin C, uric acid and glutathione (Re *et al.* 1999). Low-quality nutrition during development reduces the formation of enterocytic lipoproteins, which are required for the assimilation of dietary lipophilic antioxidants (Nutting *et al.* 2002). This may explain why poor neonatal nutrition reduces adult concentrations of these antioxidants (Blount *et al.* 2003). Poor early nutrition may also have long-term adverse effects on the uptake of other lipophilic antioxidants, such as flavonoids, and other dietary antioxidants that require intestinal membrane proteins, such as vitamin C (Rose & Wilson 1997). Thus, although there are limitations to the interpretation of *in vitro* antioxidant capacity assays (Huang *et al.* 2005), they are probably more representative of antioxidant defence status than measures of specific antioxidants. It is perhaps reassuring that an aggregate measure of antioxidant capacity, such as this, indicates the same pattern as levels of specific antioxidants have done previously (Blount *et al.* 2003).

Nestling growth and compensatory growth were closely associated in experiment 1. Birds that were reared on a LQ diet in experiment 1 generally compensated for reduced neonatal growth, so that as adults they were indistinguishable from birds that had been reared on an HQ diet. Nutritional deficit and subsequent compensatory growth may both produce deleterious effects on adult phenotypes, although, as in experiment 1, it is often unclear which is more important (Metcalfe & Monaghan 2001). Changes in both the nutritional environment and growth trajectory during development have been implicated in a number of physiological problems in later life and some mechanisms have been proposed that may explain how growth compensation can impair physiology (Metcalfe & Monaghan 2001; Metcalfe & Monaghan 2003). For example, fast growth can result in "jerry-built" bodies, in which the quality of a structure is traded-off for developmental

speed, resulting in developmental errors or weaknesses (Arendt 1997; Blanckenhorn 2000; Metcalfe & Monaghan 2003). Furthermore, organs may be "metabolically programmed" by an initial developmental environment (Waterland & Garza 1999), which in the case of nutritional restriction can involve a reduction in cell numbers (Pitts 1986). Subsequent rapid growth may then disrupt cell function, impose excessive demands on a limited cell mass (Ozane & Hales 2002) and increase oxidative stress at a cellular level (Rollo 2002). Additional experiments that control growth rates during different developmental periods would be required to disentangle the influence of slowed growth and subsequent compensatory growth on adult antioxidant capacity.

Antioxidant capacity was not directly related to either growth during the period of dietary manipulation or post-treatment growth in experiment 2. However, the fact that the highest antioxidant capacities were found in those LQ birds that remained relatively light as adults (i.e. did not compensate) suggests that growing fast either during or following the nutritional deficit carried costs. Interestingly however, even LQ birds that compensated did not have antioxidant capacities that were abnormally low (i.e. lower than HQ birds). All the experimental offspring actually faced two changes of nutritional environment during their development: one during the period of dietary manipulation (beginning day 0) and one during the post-treatment growth (beginning day 20). It is possible that the metabolism and organs of HQ chicks were programmed for their pre-hatching nutritional environment (Desai & Hales 1997; Lucas 1998). The change in environment upon hatching and the attendant alteration of growth trajectory conceivably may have produced long-term effects on the oxidant and antioxidant status of these HQ birds. The individuals with seemingly the best antioxidant defences after 17 months were those that grew relatively slowly throughout their development and showed few deviations in growth trajectory.

An additional complication is that antioxidant data is difficult to interpret (Beckman & Ames 1998). A high antioxidant capacity may indicate better levels of innate protection, which is how I have approached the data thus far. However, antioxidant defences can be induced in response to oxidative stress (Dillon & Wu 1990). High antioxidant capacity may consequently reflect high underlying levels of oxidant generation and therefore a greater need for antioxidant defences (Beckman & Ames 1998). Future studies would benefit from simultaneous assays of antioxidant capacity, oxidant production and oxidative stress (Huang *et al.* 2005).

Antioxidant defences can alter with age, although the results of previous studies have been conflicting (Beckman & Ames 1998; Masoro & Austad 2001). In this study, overall the older birds from experiment 2 possessed higher antioxidant capacities than the younger birds in experiment 1. Although this comparison needs to be interpreted with caution because it deals with two different sets of birds and different nutritional regimes, this suggests that the antioxidant defences of zebra finches increase with age, at least between the ages of 4 and 17 months. Birds were sampled at similar ages in Blount *et al.* (2003) (99.0 ± 0.2 days) and experiment 1 (130.6 ± 1.7 days). In experiment 2, any initial, negative effect of restricted early growth on antioxidant capacity in early adulthood may have been clouded by age-related changes in antioxidant assimilation and production, and oxidant production, once birds reached 17 months of age.

The free radical theory of ageing predicts that, everything else being equal, animals with lower antioxidant defences should exhibit shorter life spans (Beckman & Ames 1998). However, I found no association between antioxidant capacity and survival in experiment 1. Nor was survival linked to neonatal diet or growth. Birkhead *et al.* (1999), in contrast, found that adult males that had experienced a low-quality neonatal diet had significantly higher mortality than those males reared on a high-quality diet. However, the diets employed in that study were qualitatively similar to those used in my second experiment (i.e. more extreme than the diets in experiment 1) and were provided for 30 days (Birkhead *et al.* 1999). The number of deaths among birds in experiment 2 is currently too low to allow an assessment of the relationship between survival and antioxidant capacity at 17 months old.

Male sexual ornaments have been hypothesised to honestly advertise the bearer's antioxidant defence capacities, and therefore his susceptibility to oxidative stress (von Schantz *et al.* 1999). However, this study found no association between male bill colour and antioxidant capacity, and thus no evidence that the zebra finch bill is a condition-dependent signal of one aspect of defence against oxidative stress. The zebra finch bill, though, is an ornament based on carotenoids, which comprise only a small proportion of plasma antioxidants. As this study measured antioxidant capacity, I was therefore unable to ascertain whether bill colour was associated specifically with plasma concentrations of carotenoids. Certainly, in males that do not experience a nutritional deficit during development, bill colour does appear to reflect circulating levels of carotenoids (Blount *et al* 2003). In birds that experience

nutritional stress, this relationship appears to be uncoupled. A similar lack of correlation between antioxidant capacity and bill colour was found here, but this was the case across every diet group.

Much remains to be learnt about how developmental nutrition affects antioxidant defences in adulthood. It is possible that periods of both nutritional deficit and of growth compensation, in which animals grow at a different rate than they do on a "normal" growth trajectory, may impair adult antioxidant capacity. However, the relative importance of each is unclear, as are the mechanisms that might underlie these effects. Furthermore, the effects on antioxidant capacity resulting from an animal's developmental environment may depend on the age at which the animal is sampled.

Chapter 8

Fast growth makes slow learners: compensatory growth impairs adult cognitive performance.

ABSTRACT

Poor cognitive function in later life has been linked to early nutritional deficit. The impairment of song learning by nutritional restriction in songbirds highlights this association. However, changes in growth trajectory subsequent to a nutritional deficit can themselves carry long-term costs. Thus both the period of poor nutrition and later compensatory growth could potentially underlie poor performance in adulthood. The effects of compensatory growth and nutritional deficit have generally not been separated. I introduced a period of poor nutrition in the early growth of sibling pairs of a small vertebrate (the zebra finch *Taeniopygia guttata*). This enabled me to examine whether cognitive performance in adulthood was related to either reduced growth during nutritional deficit, or the degree of subsequent growth acceleration. As expected, song learning performance was diminished in birds experiencing a nutritional deficit. However, I found that performance on an associative learning task was linked to the degree of compensatory growth, rather than the nutritional deficit itself. Birds that exhibited fast growth following a nutritional deficit, relative to their siblings, performed poorly in the learning task. This demonstrates an important long-term cost of compensatory growth during development.

INTRODUCTION

The nutritional conditions experienced at any stage during ontogeny, from conception through to independence, can have long-term, permanent fitness consequences for animals (Lindström 1999). Offspring traits related to both survival and fecundity, for example, are affected detrimentally by both an adverse prenatal (Barker *et al.* 1993; Gorman & Nager 2004) and postnatal (Haywood & Perrins 1992; Singhal *et al.* 2004) nutritional environment. If conditions improve following an episode of nutritional deficit this can give rise to a variable degree of compensatory growth wherein the organism accelerates or prolongs their growth and attains normal, or near normal, adult size and morphology (Arendt 1997). It has recently been recognised that such compensatory growth appears itself to carry a variety of long-term costs (Eriksson *et al.* 1999; Forsén *et al.* 1999; Jennings *et al.* 1999; Metcalfe & Monaghan 2001; Hales & Ozanne 2003; Ozanne & Hales 2004; Singhal & Lucas 2004). Thus both the period of poor nutrition and the subsequent growth changes could potentially underlie the later poor performance. Despite the obvious importance of these processes for our understanding of the adaptive significance of different developmental patterns (Bateson *et al.* 2004), the effects of compensatory growth and nutritional deficit have generally not been separated (Metcalfe & Monaghan 2003).

Nutritional deficit impairs the development of different organs to varying extents and often the development of some organ systems is prioritised over others. It has been suggested that cognition is protected against developmental stress to a greater extent than other body functions under a process of 'brain sparing' (Ravelli *et al.* 1998). Yet many recent studies of human infants have provided evidence that sub-optimal nutrition in early life can reduce neural development (Fernstrom 2000; Wainwright 2002) and subsequent intelligence quotients (Lucas *et al.* 1992; Smart 1993; Lucas *et al.* 1998; Scrimshaw 1998). Together with evidence from animal studies (Smart 1986), this suggests that developmental stress acting during critical stages of neurogenesis can indeed have permanent adverse effects on cognitive function.

There has been considerable recent interest in the effect early nutrition can exert on brain development in birds. However, this interest has centred invariably on the development of the song centres of male brains. Nowicki *et al* (1998) developed the

idea of the nutritional stress hypothesis, which suggests that females can utilise learnt features of male song as indicators of mate quality. The areas of the brain that control song learning, principally the high vocal centre (HVC), develop primarily during the postnatal juvenile period (Alvarez-Buylla *et al.* 1992). Thus they develop during a time when birds are growing rapidly (Ricklefs 1983) and are particularly vulnerable to nutritional deficit (O'Connor 1984). The hypothesis proposes that nutritional quality during this sensitive window, in tandem with heredity (Airey *et al.* 2000), determines song centre development and therefore the proficiency with which song is learnt. Many aspects of male phenotype and fitness are affected by development. However, song performance is a trait that is easily advertised and may indicate a number of male attributes to females. For example, song quality may reflect a male's overall phenotypic condition as a result of its developmental environment (Searcy *et al.* 2004), which in turn is likely to affect its parenting ability (Buchanan & Catchpole 2000) and lifespan (Birkhead *et al.* 1999). In addition song may indicate underlying heritable aspects of parental care (Nowicki *et al.* 1998) and also genotypic differences in responses to nutritional stress (Williams 1966). There is now considerable evidence to support the nutritional stress hypothesis (Nowicki *et al.* 2000; Nowicki *et al.* 2002; Buchanan *et al.* 2003; Spencer *et al.* 2003; Buchanan *et al.* 2004), even though some controversy still remains (Gil *et al.* 2004).

It is understandable that avian studies have focussed on song learning because of the role of male song as a sexually selected trait (Searcy & Andersson 1986; Catchpole 1987). Song learning in early life, which can be modulated by nutritional conditions, is likely to be important in determining adult male reproductive success (Reid *et al.* 2004) and therefore fitness. However, while singing impairment following a neonatal nutritional deficit clearly does occur, it is not clear whether the observed deficit in song learning is in fact one component of a more general learning impairment. Cognitive performance in general could obviously have a profound effect on lifetime fitness, in terms of foraging performance, mate acquisition, parenting, as well as in its integral role in physiological homeostasis. Nor is it clear whether the observed effects on song learning are a direct consequence of the nutritional deficit, or attributable to subsequent compensatory growth induced by improved conditions following the deficit period. Here I manipulated the nestling diet of zebra finches, *Taeniopygia guttata*, and

examined the effects of neonatal diet quality on both song learning performance and performance on an associative learning task in adulthood. My experiment was designed to allow a comparison between siblings from different dietary backgrounds. I measured growth rates both during and after the diet treatment, enabling me to examine associations between growth rate and both song learning and more general cognitive performance.

METHODS

Early nutritional deficit

In order to compare individual performance in relation to early growth, while allowing for genetic effects, I compared only genetic siblings of the same sex reared in broods given different diet treatments. Zebra finches with previous breeding experience were randomly paired and allowed to nest in individual breeding cages (60 cm x 45 cm x 40 cm). Prior to their chicks hatching, pairs were kept on a 12:12 light dark cycle and fed on a standard diet. The standard diet consisted of *ad lib* mixed seed (foreign finch mixture, J. E. Haith, Cleethorpes, UK), water, plus 5g of rearing and conditioning supplement (J. E. Haith, moistened at a 3:2 mass ratio with Daily Essentials 2 supplement (The Birdcare company, Nailsworth, UK), which itself had been diluted 1g/litre with water), three times per week. When the first chick in each nest hatched, nests were allocated randomly to one of two treatment groups, one of which involved a period of early nutritional deficit. Nests in this treatment were given a diet that consisted only of *ad lib* mixed seed and water, but no further dietary supplements (i.e. low quality (LQ) diet). The other group were given the diet normally given to pairs rearing chicks; this included, in addition to *ad lib* seed, cuttlefish plus 10g of Haith's protein supplement/homogenised egg mix plus 15ml of Daily Essentials 2 supplement (dilution of 1g/litre) every day (i.e. high quality (HQ) diet). Details of the protein and nutrients that each diet provided are provided in chapters 6 and 7 (the diets were identical to those detailed in experiment 2 of chapter 7). Since I did not know the sex of chicks at hatching, I ensured that same sex siblings experienced different dietary regimes by

using the following experimental protocol. I moved all chicks in each brood on the day of hatching to different families in the two dietary treatment groups. After chicks had been moved, each brood consisted of 3 unrelated chicks. This procedure therefore also controlled for any effects of fostering, since no chicks were reared by their genetic parents. The deficit treatment lasted for the 20-day nestling period, after which all fledglings received the same standard diet as had been given to their parents prior to hatching (see above). Each chick was weighed on the day of hatching and at 20 and 70 days post-hatching, and mass increments for the period of nutritional deficit (day 0 to 20) and the post-treatment period (day 20 to 70) were calculated. By 70 days post-hatching, chicks have reached their full size (Boag 1987). Offspring were removed from their foster mother at 35 days post-hatching and placed in single sex groups of 4-5 birds.

Song learning

The song learning performances of brothers that received opposite diet treatments (n = 7 pairs of brothers) were compared in the following way. Male zebra finches learn their adult song from a single tutor (Slater *et al.* 1988) during a sensitive period lasting from 35 to 90 days post-hatching (Tchernichovski *et al.* 2001). Foster fathers were therefore removed when the oldest chick in their nest reached 15 days to avoid young males learning from them. When placed together in the groups of 4 or 5 on day 35, full-sibs were always placed together in the same group and therefore pairs of brothers were provided with the same song tutor. Tutors sang songs with 6 to 9 different elements, which was the upper range in our aviaries. Each song tutor was housed with his female partner and separated from the young birds by a mesh partition. Tutors were removed when experimental birds were reached adulthood (90-100 days old).

The song of the seven pairs of brothers was recorded (using an Aoi ECM-1025 microphone and Sony TCM-20DV recorder) by placing each young male alone in a cage, with the tutor's female partner in an adjacent cage. Song was recorded on two occasions, when males were 114 ± 1 day old and when they were 150 days old. Song recordings were then transferred to sonograms (using GW Instruments Soundscope package, GW Instruments, Inc., Massachusetts, USA), from which 118.72 ± 18.66 phrases per male were analysed. Males sing a repeated song phrase, which is

stereotyped, and consists of sub-units known as elements (or syllables). These elements are distinguished from each other by time gaps or amplitude changes and occasionally the same element is repeated within a phrase. Because female zebra finches are attracted to males with a large number of elements in their repertoire (Clayton & Pröve 1989), I recorded the number of different elements contained within each male's phrase.

General learning performance

Offspring of both sexes were given an associative learning task when they were adults (i.e. approximately 150 days old). These trials were conducted in a circular foraging arena (height 40cm, diameter 150cm). The arena contained seven corridors leading from a central area. At the end of each corridor was a screen behind which seed was hidden. A wire mesh, pulley-operated, central holding chamber kept the bird in the centre of the arena during acclimation phases. Initially, birds were given pre-testing shaping trials to ensure that they could locate the food hidden behind the screens. The birds were then required to learn an association between the colour of a screen and the presence of food. Each bird was given eight consecutive trials per day, for two consecutive days, before the first feeding in the morning. During these 16 trials, only one of the screens, coloured yellow, had food behind it; the other six screens were black. Each trial consisted of a 10-minute acclimation period inside the holding chamber, then a search phase. During the search phase, the number of errors (i.e. the number of different unrewarded screens visited) a bird made prior to finding the rewarded tray was recorded. Once the bird located the rewarded tray it was allowed to feed for 30 seconds, then removed from the arena. Once a bird had visited at least one screen within 20 minutes it was allowed to continue foraging for as long as it took to find the food. In some trials a bird did not forage and therefore visited none of the screens within 20 minutes. When this occurred the association was reinforced by swivelling the rewarded food tray around so that the food could be seen, in front of the yellow screen, from the centre of the arena. The bird was then allowed to feed for 30 seconds as usual. Following each feeding from the rewarded screen, a bird was subjected to a 10-minute retention interval prior to the start of the next trial. The location of the rewarded yellow screen was randomly altered between trials. The zebra finch is a size-monomorphic species (Zann 1996) and there

were no sex differences in growth rates either during treatment (*t*-test: $t_{32} = 0.81$, $P = 0.42$) or post-treatment (day 20 to 70) ($t_{32} = 1.29$, $P = 0.21$). I therefore pooled the pairs of brothers (n = 7) and pairs of sisters (n = 10) for analysis of performance in the associative learning task.

RESULTS

Siblings allocated to different diet treatments did not differ in hatching mass (LQ birds = 1.0 ± 0.03g; HQ birds = 1.0 ± 0.04g; paired t-test: $t_{16} = 1.16$, $P = 0.26$). Comparing body mass at day 0, 20 and 70 post-hatching shows that the mass increments differed between the diet treatment groups (repeated measures ANOVA; age: $F_{2, 64} = 1596.33$, $P < 0.001$; nestling diet: $F_{1, 32} = 5.60$, $P = 0.02$; age x diet: $F_{2, 64} = 5.00$, $P = 0.01$). Nutritional deficit slowed the nestling growth of LQ birds relative to their HQ siblings (the instantaneous growth rate of LQ birds between 0 and 20 days post-hatching was, on average, 4.9% lower than their HQ siblings; paired *t*-test: $t_{16} = 2.70$, $P = 0.02$). By the end of the diet treatment (20 days post-hatching), offspring in the LQ group were on average 13.4% lighter (11.6 ± 0.40g) than those in the HQ group (13.4 ± 0.26g). In the post-treatment period (day 20 to 70 post-hatching, all birds now on the same diet) LQ birds gained an additional 46.50 ± 4.78 % of their day-20 body mass, compared to a 29.49 ± 2.91 % average gain in the HQ birds ($t_{16} = 3.07$, $P = 0.01$). By day 70 post-hatching, birds were effectively fully-grown and LQ birds were on average only 3.4% lighter (16.8 ± 0.46g) than their HQ siblings (17.4 ± 0.52g).

However, there was much variation within the treatment groups in the extent to which growth was slowed in response to the dietary regime, and in the degree of catch-up growth that occurred following resumption of normal feeding (coefficient of variation of within-sibling-pair differences in growth between 0 and 20 days = 75.5% and between 20 and 70 days = 90.6%). Furthermore, the LQ birds that showed the strongest reductions in growth during the deficit period, relative to their HQ siblings, were not the same birds that produced the most extreme compensatory responses once the deficit period ended (correlation between sib differences in day 0 to 20 growth and day 20 to 70 growth: $r_{15} = -0.31$, $P = 0.23$). This allowed me to separate the effects on

cognitive abilities of the slowing of growth during the period of nutritional deficit from effects of later compensatory growth.

Figure 8.1 The mean number of errors made on the associative learning task by experimental birds. All birds (i.e. both sexes and birds from both diet treatment groups) are included. The dotted line indicates the number of errors expected by chance. The dashed line denotes a 24-hour retention interval (i.e. trial 9 was the first trial of day 2).

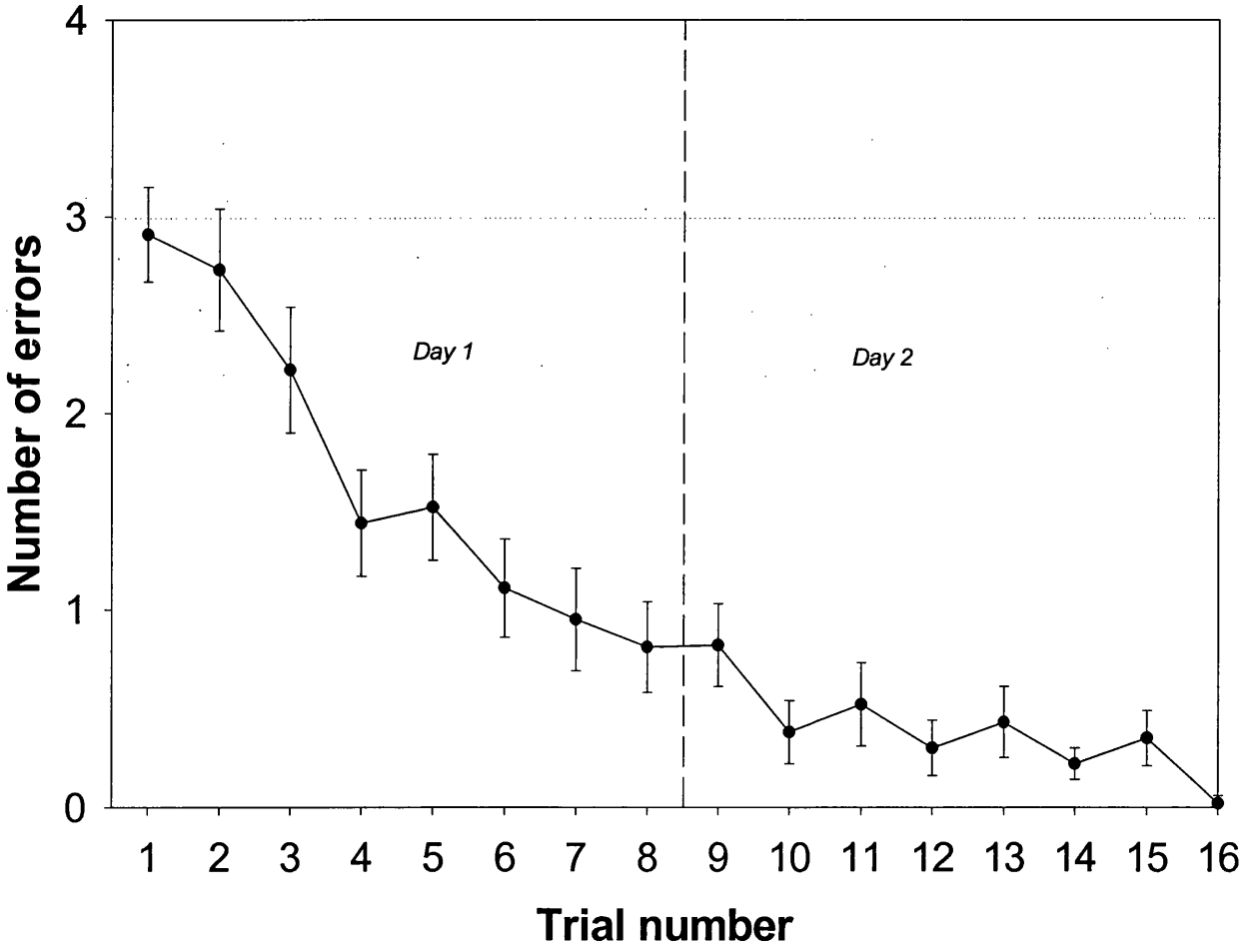


Figure 8.2 Relationship between growth rate following a period of low quality nutrition and learning performance in adulthood. Each data point represents a sibling pair. Within-sibling differences (LQ sib – HQ sib) in growth rate and learning performance are plotted on the horizontal and vertical axis, respectively. Growth rate is the instantaneous growth rate from day 20 to 70, following the period of nutritional deficit. Positive values indicate that the LQ sib grew faster, post-treatment, than its HQ sib. The learning performance of the birds was ranked on the basis of the number of trials it took them to achieve two without error, with those not reaching this criterion getting a score of zero; the fastest learners thus have the highest score. The figure shows the difference between the sibling pairs, and negative values mean that the LQ sib had a poorer learning performance than its HQ sib.

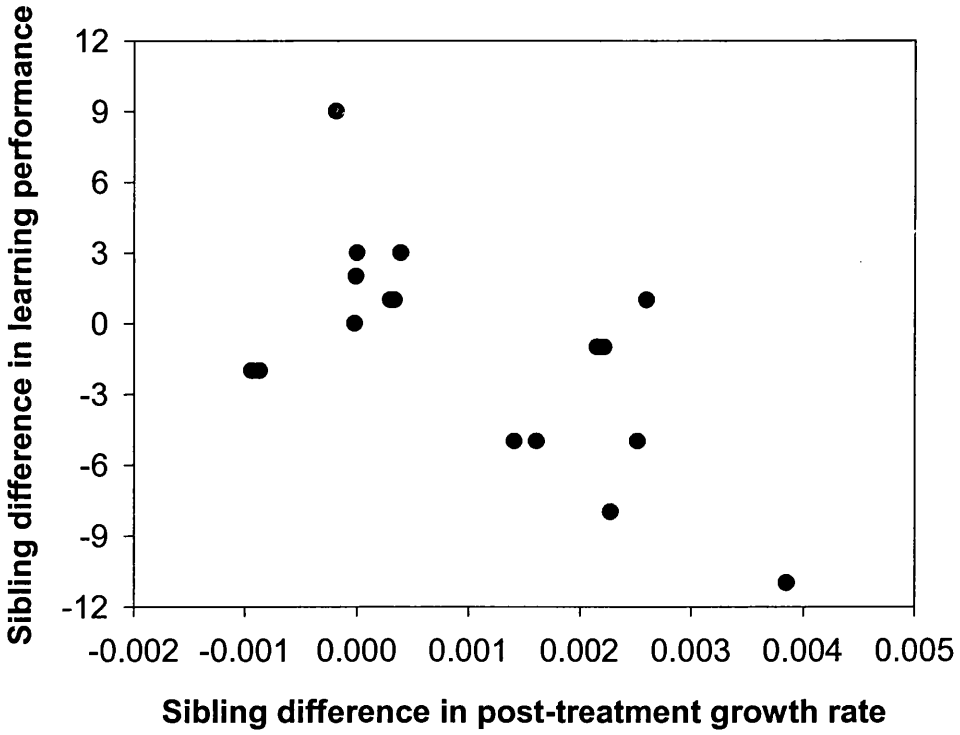


Table 8.1 Factors affecting the number of errors made in the associative learning task. Statistics are from a mixed model where trial number is a repeated-measure within bird identity (random effect) and using binomial error distribution. Family of origin was included as a random factor. Sex and diet were included as fixed factors. Asterisks indicate statistical significance.

	Test statistic	Parameter estimate (± SE)
Bird identity	$Z = 0.13$	0.016 (0.126)
Trial № (nested within bird identity)	$Z = 14.07$***	0.988 (0.070)
Family of origin	$Z = 1.12$	0.132 (0.118)
Offspring sex	$F_{1,13.4} = 0.05$	-0.063 (0.269)
Diet treatment	$F_{1,13.7} = 0.74$	-0.172 (0.200)

*** $P < 0.0001$

Differences between the pairs of brothers in the number of different song elements produced as an adult male were associated with the differences in their mass increment from day 0 to 20 (multiple regression: $F_{1,4} = 7.27$, $P = 0.05$), but not with differences in their mass increment from day 20 to 70 ($F_{1,4} = 0.49$, $P = 0.52$). The slower the LQ birds grew during the treatment period, relative to their HQ siblings, the fewer elements they incorporated into their song. Males that experienced normal nutrition during early growth sang a greater number of different elements per phrase (5.71 ± 0.47) than their brothers who experienced an episode of nutritional deficit on the LQ diet (4.43 ± 0.48) (paired *t*-test: $t_6 = 2.47$, $P < 0.05$). This negative effect of early nutritional deficit on song learning ability is as reported in other studies (Nowicki *et al.* 2000; Spencer *et al.* 2003).

In the associative learning task, birds generally reduced the number of errors they made over the course of the 16 trials (figure 8.1). Associative learning performance did not differ with sex or family of origin (table 8.1) or foster family in which the experimental birds were reared ($F_{18, 15} = 0.56$, $P = 0.88$). The associative learning performance, however, while not specifically related to the diet treatment (table 8.1),

was strongly related to the extent to which growth compensation occurred when normal nutrition was provided (multiple regression; sibling differences in the mass increment from day 20 to 70: $F_{1,14} = 8.03$, $P = 0.01$; sibling differences in the mass increment from day 0 to 20: $F_{1,14} = 0.02$, $P = 0.90$). LQ birds that showed a strong compensatory growth response relative to their HQ sibling performed relatively poorly on the associative learning task (figure 8.2).

DISCUSSION

The impairment of song production in adult birds has been attributed to a nutritional shortfall at a time when the song learning centres are forming in the brain (Nowicki *et al.* 1998). My results support this nutritional stress hypothesis. Males receiving qualitatively poor neonatal nutrition possessed fewer elements in their adult song repertoires than their brothers who had received a superior nestling diet. More significantly however, these results demonstrate a more general cognitive impairment stemming from poor developmental conditions. However, this impairment was associated with the degree of compensatory growth undertaken following early nutritional deficit, rather than the nutritional deficit itself. This is likely to have far reaching consequences for adult performance in many circumstances.

Whilst the size of the avian song centre is known to be reduced by nutritional deficit (Buchanan *et al.* 2004), this is the first experimental demonstration of a link between growth strategies and more general cognitive performance in birds. Compensatory growth is known to produce various costs in adulthood (Metcalfe & Monaghan 2001), but to my knowledge, this is the first time that it has been implicated in the impairment of cognitive function. In some species, such as the zebra finch, investment in body size and sexual attractiveness is a priority, especially if they are short-lived and may only have a single mating opportunity in their lifetime (Birkhead *et al.* 1999). Individuals attempting to recover from a poor start will have finite resources to invest in such compensation. In such cases, animals may trade-off the development of particular structures, perhaps resulting, in this example, in proportional under-

investment in the brain. In other species, conversely, 'brain sparing' might be a priority (Ravelli *et al.* 1998).

These results raise intriguing questions about how growth rate in early life may impinge on later adult performance. One possibility is that fast growth represents a stressor. Prolonged stress during a compensatory period of growth may produce chronic levels of corticosterone (CORT). Long-term exposure to CORT can result in irreversible damage to the hippocampus, a brain region closely associated with learning and memory (Kim & Yoon 1998). The vertebrate brain also contains a large number of polyunsaturated fatty acids, which are especially susceptible to free radical damage (Coyle & Puttfarcken 1993). An increase in free radical production during relatively fast growth periods may thus result in oxidative damage within the brain (see von Schantz *et al.* for a brief review). Another possibility is that accelerated growth trajectories reduce telomere length and thereby hasten cell death. Postnatal compensatory growth in rats reduces telomere length in kidneys, although not in brain cells (Jennings *et al.* 1999). Alternatively compensatory growth may be achieved by overgrowth of a limited cell mass, which could result in a poorly functioning organ (Pitts *et al.* 1986). Although it is speculative to discuss these potential underlying mechanisms, there obviously exists much scope for further experimental study.

The results of this study highlight the importance of considering not only the effects of an early nutritional deficit, but also the effects of subsequent growth on adult performance (Metcalfe & Monaghan 2001; Metcalfe & Monaghan 2003). Future studies should consider examining the relative importance in determining adult phenotype of periods of restricted growth and periods of accelerated growth. Empirical approaches may be able to incorporate control groups of animals that experience sustained nutritional restriction, in addition to groups that are allowed to undergo compensatory growth following a nutritional deficit.

Several recent human studies have linked accelerated growth in infancy with an increased risk of obesity (Waterland & Garza 1999), insulin resistance (Yudkin & Stanner 1998) and cardiovascular disease (Eriksson *et al.* 1999; Forsén *et al.* 1999) in later life. The results of this study reveal a previously unreported connection between compensatory growth and adult cognitive performance. This study thereby provides empirical support for the suggestion that fast growth during development can be

detrimental in the longer term and has potentially fundamental implications for clinical nutrition policy, and for studies of both developmental plasticity and life history trade-offs (Bateson *et al.* 2004).

Chapter 9

General discussion

Mate discrimination and preferences

It is now established that animals can discriminate between potential mates that vary in their reproductive value (Parker 1983; Andersson 1994). Mating preferences for particular traits that indicate a mate's value may thus evolve (Kirkpatrick & Ryan 1991). Generally females are expected to be the choosier sex because they tend to invest more in gamete production and parental care and their costs of breeding will thus be higher (Kokko & Monaghan 2001; Kokko & Johnstone 2002). Males should therefore gain benefits from signalling to females their genetic quality, condition and resources, and competing for access to these mates, which represent to them a limiting resource. Consequently, selection is likely to be stronger on female, rather than male, mating preferences, but stronger on male, rather than female, sexual signals (Andersson 1994). However, different breeding systems will vary in the levels of breeding investment made by males and females (Reynolds 1996). When females are offered no resources other than sperm in polygamous breeding systems such as leks, males are expected to evolve conspicuous signals to advertise their genetic quality, but they will display no choosiness (Kirkpatrick & Ryan 1991; Andersson 1994). Contrastingly, in socially monogamous species with biparental care, parental investment and the costs of breeding often differ little between the sexes. Mutual mate choice can arise in such breeding systems, with the result that both males and females exhibit mating preferences (Johnstone *et al.* 1996; Kokko & Johnstone 2002).

There is an inveterate tendency to consider these mating preferences as species-specific and uniform across a population. Often observed variation in preferences has been attributed to mate assessment errors or to the poor methodology of investigators (Widemo & Sæther 1999). However, it is increasingly apparent that mating preferences can vary appreciably between individuals of the same species and within the same population (Jennions & Petrie 1997; Widemo & Sæther 1999). In this chapter, I briefly review the mating preferences exhibited by zebra finches,

Study	Main interest	Status of mating options	Status of choosers	Number of mating options	Test length (min)	Trait(s) preferr BC	SR
ten Cate & Mug (1984)	Imprinting	?	N	2	40	-	**Yes**
Burley & Coopersmith (1987)	BC	N	N*	4	180	**Yes**	-
Houtman (1992)	EPCs	?	?	8/9	60	Yes	**Yes**
Wynn & Price (1993)	Condition	> 4 months	> 4 months	2	> 100	-	-
Collins *et al.* (1994)	BC & SR	?	?	2	50	No	**Yes**
Sullivan (1994a)	BC	N (> 3 months)	N (> 3 months)	4	120	No	-
Collins (1995)	Experience	N & E	N	2	30	-	**Yes**
Vos (1995)	Imprinting (BC)	?	N (> 4 months)	2	20	No	-
de Kogel & Prijs (1996)	Brood size	N (< 1 year)	N (6-18 months)	2	20	**Yes**	Yes
Balzer & Williams (1998)	Reproductive effort	?	E (> 6 months)	6	30	No	**Yes**
Blount *et al.* (2003)	BC	N (< 1 year)	E	2	130	**Yes**	-
Forstmeier (2004)	SR & BC	N** (333 ± 78 days)	N (314 ± 96 days)	1	5	No	**Yes*** **
Forstmeier & Birkhead (2004)	Repeatability	N (> 6 months)	N (> 6 months)	4	180	No	**Yes**

Table 9.1 Studies that have examined the mating preferences of female zebra finches. Traits that explained a significant amount of variation in mating preferences are labelled "Yes". Where more than one trait affected preferences, the most influential trait is highlighted in bold. Dashes denote that the trait was not measured. "Test length" encompasses the total length of time that choosers had to assess potential mates (i.e. acclimation periods + recording periods). SR = song rate; BC = bill colour; M = body mass; N = naïve (i.e. no previous breeding experience); E = previous breeding experience. * Females were pre-exposed to males; ** Experimental males were allowed to court females for 5 days prior to tests as practice; *** Song rate *per se* did not affect female preference, but the presence or absence of song did.

Taeniopygia guttata, in previous studies and in my own experiments. I attempt to explain why the results of these studies vary and, in particular, I discuss the variation in preferences that can be observed between individuals within the same experiment or population.

Zebra finch mating preferences: variation between studies

Numerous studies that have examined the mating preferences of female zebra finches in relation to male morphological traits (table 9.1) and several, but fewer, studies have investigated male mating preferences (for example, Burley & Coppersmith 1987; Wynn & Price 1993; de Kogel & Prijs 1996; Monaghan *et al.* 1996; Jones *et al.* 2001). Females show preference functions for high song rate (for example, Collins *et al.* 1994), red bill colour (for example, Burley & Coopersmith 1987), good body condition (Wynn & Price 1993) and complex songs (Clayton & Pröve 1989). As table 9.1 shows, the criteria upon which females apparently base their selection of a mate vary between studies and the relative importance of song rate and bill colour has been especially controversial. However, when these two traits have been examined simultaneously, song rate generally appears to be a more important mate choice cue for females than bill colour (table 9.1; also see Collins & ten Cate 1996).

The method by which song rate is estimated is crucial to the outcomes of mating preference studies (Forstmeier 2004). Studies that measure "song rate" during preference tests may produce spurious associations between this measurement and male attractiveness if they measure female preference in terms of affiliation behaviour, because the presence of a female stimulates males to sing (e.g. ten Cate & Mug 1984; Collins *et al.* 1994, experiment 1; de Kogel & Prijs 1996). Experiment 2 of Collins *et al.* (1994) did record song rate outside preference tests, but suffered from pseudoreplication. Thus only Houtman (1992) and Forstmeier & Birkhead (2004) have accurately shown that intrinsic male song rate, measured independently of preference tests, influences female preference. In my experiments, I found no evidence that females preferred males with either a high intrinsic song rate or a high song rate during preference tests (see chapters 5 and 6). I will discuss shortly some potential explanations for the contradictory results produced by studies of song rate.

Male song rate and bill colour have been proposed to be condition-dependent traits (Birkhead *et al.* 1998) that provide information about a male's reproductive

value. These traits may reflect a male's current condition and the "quality" of genes that his offspring would inherit (Andersson 1994). Indeed, both song rate (Houtman 1990; Collins *et al.* 1994) and bill colour (Price & Burley 1993; Price 1996) appear to have a heritable component. These traits may also indicate male mating status, because bill colours are less red and song rates are lower in breeding birds (Collins *et al.* 1994). However, the evidence for the condition-dependence of song rate in particular is inconsistent. Song rate has been hypothesised to be antioxidant-dependent (von Schantz *et al.* 1999), but the metabolic costs of song are low (Oberweger & Goller 2001; Ward *et al.* 2004). Previous studies have employed different measures of "condition" (table 9.2), but song rate was condition-dependent in only one case (Birkhead *et al.* 1998). The evidence that male bill colour reflects condition is more convincing. Bill colour was positively associated with body condition in two out of three previous studies that reported correlation statistics (table 9.2). Furthermore, males with relatively red bills show a stronger cell-mediated immune response (Blount *et al.* 2003). Bill colour and song rate were positively associated in 3 out of 5 studies that reported this statistic. In one of these studies song rate was calculated during preference tests; therefore this relationship is likely to be an artefact because females affiliated most often with, and were therefore sung to more often by red-billed males (de Kogel & Prijs 1996). Even when significant correlations exist, they are not strong, and require a large sample size to reveal them (table 9.2). I measured song rate independent of preference tests and calculated condition as size-adjusted mass (as in de Kogel & Prijs 1996). Across my experiments, there was a significant combined probability of bill colour, but not song rate, being condition-dependent (table 9.3). The combined probability of song rate and bill colour being associated was non-significant (table 9.3). Such an inconsistent relationship between bill colour and song rate suggests that they may convey different information (e.g. different aspects of condition) to females (Birkhead *et al.* 1998). An additional confusion is that when bill colour appears condition-dependent, it may not necessarily influence female preference (Forstmeier & Birkhead 2004) and when it is found not to be condition-dependent, females may still prefer red bills (de Kogel & Prijs 1996). In summary, the degree to which song rate reflects male condition remains uncertain. This is true to a lesser extent in the case of bill colour, but the relative importance of both of these male traits as female mate choice cues is ambiguous.

Table 9.2 Correlations between male song rate, bill colour and condition in previous zebra finch studies. Condition = [a] body mass adjusted for body size, [b] capture order, [c] body mass.

Study	Song rate & bill colour	Song rate & condition	Bill colour & condition
Houtman (1992)	$r_{20} = 0.45$*	-	-
de Kogel & Prijs (1996)[a]	$r_{50} = 0.44$**	$r_{50} = 0.16$	$r_{50} = 0.06$
Birkhead *et al* (1998)[b]	$r_{s29} = 0.08$	$r_{s29} = 0.44$*	$r_{s29} = 0.43$*
Forstmeier (2004)[c]	$r_{102} = 0.20$*	-	-
Forstmeier & Birkhead (2004)[c]	$r_{102} = 0.04$	$r_{102} = 0.15$	$r_{102} = 0.26$**

*$P < 0.05$

**$P < 0.01$

Table 9.3 Correlations between song rate, bill colour and condition in my experiments. Song rate was calculated independently of mate preference tests. Body condition was calculated as the standardised residual of body mass regressed on body size components. Body condition and mass were interchangeable in each case and produced qualitatively the same results. The ages at which measurements were taken are listed in the relevant chapters (except chapter 8; birds from this experiment were 150 days old when measured). Linear ($P > 0.104$) and quadratic models ($P > 0.061$) of female bill colour and condition were non-significant in all cases except the experiment in chapter 2 (here a linear model ($P = 0.007$) was more significant than a quadratic model ($P = 0.010$)). Combined probabilities (Sokal & Rohlf 1995) are shown in cases where all correlations were in the same direction.

	Males			
Chapter of experiment	Song rate & bill colour	Song rate & condition	Bill colour & condition	Female bill colour & condition
2	-	-	-	$r_{s56} = 0.32$*
5	$r_{s27} = 0.02$	$r_{s27} = -0.46$*	$r_{s27} = 0.14$	$r_{s22} = 0.26$
6	$r_{s18} = 0.05$	$r_{s18} = 0.27$	$r_{s18} = 0.47$*	$r_{s18} = 0.26$
8	$r_{s23} = 0.48$*	$r_{s23} = 0.38$	$r_{s23} = 0.43$*	$r_{s30} = 0.22$
Combined probability	$\chi^2_6 = 8.84$	-	$\chi^2_6 = 14.97$*	$\chi^2_6 = 17.12$*

* $P < 0.05$

Rather than accurately reflecting male condition, song rate may provide other information for females. Song may advertise to females that a male is motivated and ready to breed. As such, females may initially be attracted to eager, singing males. Forstmeier (2004), for example, found males that sang had much better copulatory success than silent males during 5-minute tests, but the song rate of those that sang had no influence on their success. Sullivan (1994a) prevented courtship by using one-way mirrors in preference tests and females preferred to affiliate with orange-billed males. It is possible that females expressed social preferences in this situation (Collins & ten Cate 1996). An accurate assessment of bill colour, in contrast, may require a longer assessment period. Indeed, of the three studies in which bill colour was apparently the predominant mate choice cue, two allowed relatively long assessment periods (table 9.1). Furthermore, prolonged assessment periods may allow birds to evaluate the body condition of potential mates directly and more accurately. Experiments in which body condition influenced mating preference have allowed relatively long windows for mate assessment (female preferences: Wynn & Price 1993; table 9.4; male preferences: Monaghan *et al.* 1996 (> 24 hours); Jones *et al.* 2001 (480 minutes); table 9.4). One caveat to this idea is that eventual pair formation among zebra finches seems to be decided within 30 minutes of introduction (Silcox & Evans 1982). However, by introducing two females and two males into the same cage, Silcox & Evans (1982) employed a different preference test methodology to most studies. In this situation, intrasexual competition is liable to affect the expression of mating preferences and therefore the observed mating pattern (Qvarnström & Forsgren 1998).

The use of multiple cues in mate selection has garnered attention recently (Candolin 2003; van Doorn & Weissing 2004). Female zebra finches appear to use both male song rate and bill colour as cues, although, as I have discussed previously, the evidence for this is mixed. These cues may provide independent information (van Doorn & Weissing 2004) and/or may be assigned weightings to allow the formation of an overall "index" of attractiveness (Jennions & Petrie 1997). Song complexity (i.e. the number of different elements contained within a male's song phrase) also seems to influence female mating preferences (Clayton & Pröve 1989). Song complexity reflects a male's developmental history (Spencer *et al.* 2003; chapter 8) and thus aspects of male quality (Nowicki *et al.* 1998). However, the importance of song

complexity as a female mate choice cue in relation to bill colour, song rate and body condition has yet to be examined.

Male zebra finches are believed to prefer females with intermediate bill colours (Burley & Coopersmith 1987; table 4b) and females in good nutritional condition (Wynn & Price 1993; Monaghan *et al.* 1996; Jones *et al.* 2001; table 9.4b). Thus a non-linear relationship between female bill colour and condition may be expected, but the evidence for this is weak (table 9.3). A previous study did not find that female bill colour was condition-dependent (de Kogel & Prijs 1996, $r_{62} = 0.07$). Interestingly, in my experiments, there was generally a positive, but non-significant relationship between female bill colour and condition, but a combined probability analysis suggests that the association may be significant (table 9.3). The observation that males prefer intermediate bill colours therefore remains puzzling. Intermediate bill colours are most common among very young adult females (Burley & Coopersmith 1987), which may help to explain male preferences. Males apparently prefer relatively young females (chapter 3), providing that they are not sexually naïve (chapter 5), possibly because reproductive value declines more quickly in females than males due to differential adult mortality (de Kogel 1997; chapter 7). Males also appear to imprint on the bill colour of their mother (Vos 1995), therefore variation in male bill colour preferences may in part reflect differences in early experiences, such as imprinting periods (Burley & Coopersmith 1987). Another possibility is that males may avoid females with relatively red bills because these individuals may be trading-off their potential investment in parental care for the maintenance of dominance ranking. However, I found no evidence to suggest that females possessing relatively red bills are more likely to establish dominance status (experiment from chapter 2, data not shown).

Sexual trait variation

A prerequisite of a mate choice cue is that it exhibits a level of variation that is sufficient for differences between potential mates to be detected. Sexual traits often show a greater variation than ordinary morphological traits (Alatalo *et al.* 1988) and trait variation is expected to determine the effectiveness of a cue (Reid & Weatherhead 1990). Certain sexual traits may also exhibit more variation than others;

Table 9.4 Traits of experimental birds that affected the mating preferences of (a) females and (b) males in my experiments. The mean trait differences of mating options within preference test dyads are shown (for female bill colour, the mean within-dyad difference in deviation from the population median is shown; see relevant experiments for details of these calculations). Where a trait explained a significant amount of the variation in mating preferences, it is highlighted in bold, with an indication of the direction of the preference. Body condition produced qualitatively the same results as body mass. The mate assessment periods (including acclimation periods and test recording) were as follows: chapter 2: 240 minutes; chapters 3 & 4: 200 minutes; chapters 5 & 6: 105 minutes.

(a) Female mating preferences

		Test dyad differences					
Chapter	**N**	**Bill colour**		**Body mass**		**Age**	
3	24	-		24.7 ± 4.1%	No	10 ± 1 months	No
4	48	-		23.3 ± 3.0%	**Heavier**	9 ± 1 months	**Older**
5	17	0.7 ± 0.2	No	7.8 ± 1.3%	No	39/40 months	**Older**
6	10	0.9 ± 0.2	**Red**	7.2 ± 1.9%	**Heavier**	0/1 months	No

(b) Male mating preferences

		Test dyad differences					
Chapter	**N**	**Bill colour**		**Body mass**		**Age**	
2	23	1.1 ± 0.2	No	13.0 ± 1.7%	**Heavier**	4 ± 1 months	No
3	24	-		26.0 ± 3.9%	No	10 ± 1 months	**Younger**
4	46	-		18.1 ± 1.7%	**Heavier**	8 ± 1 months	No
5	12	0.5 ± 0.1	**Intermediate**	7.3 ± 1.6%	No	39/40 months	**Older**
6	10	0.9 ± 0.2	No	15.5 ± 3.9%	No	0/1 months	No

for example, in zebra finches, song rate is thought to show twice the variation of bill colour (Houtman 1990). Benign lab conditions and domestication effects such as inbreeding may reduce trait variation and this may be one reason for the differences in the results of mating preference studies. For example, Wynn & Price (1993) attempted

to measure the bill colour of their birds, but failed to observe meaningful differences. Trait variation may be increased in harsh natural environments, or as a result of experimental manipulations of phenotypic traits or during breeding events. Body mass, for example, was generally more variable among test dyads from my breeding experiments (chapters 3 & 4) than in other experiments (see table 9.4). However, there was no discernible pattern in my results to suggest that a trait was more likely to affect mating preferences when its variation increased (table 9.4).

Zebra finch mating preferences: variation between individuals

In a recent, extensive study of female zebra finch mating preferences, individual females appeared to differ consistently in their own mating preferences, but there was little between-female agreement in preferences (Forstmeier & Birkhead 2004). One factor that may create variation in mating preferences is previous experience. It has been suggested that a preference function for a particular trait is a result of a heritable, innate predisposition and a reference template (*sensu* Widemo & Sæther 1999). These reference templates are potentially flexible and can be modified by imprinting periods (e.g. female songbirds often imprint on their father's song (Hauser 1996)). Internal standards may also be adjusted subsequent to an imprinting period, with respect to the previous experience of an individual with actual or potential mates. For example, female zebra finches appear to adjust their reference template for male song rate, at least in the short-term, following exposure to either low or high song rates (Collins 1995). Birds may need to observe how traits such as song rate, condition and bill colour relate to each other, and effectively "learn" a preference (Collins & ten Cate 1996). Preference functions may thus be adjusted as animals gain more experience and are exposed to a greater range of potential mates. My experiments produced a number of examples of experience-related mating preferences. Older, more experienced males, but not relatively young males, appeared to discriminate between females based on body mass (chapter 2) and bill colour (chapter 5). Furthermore, only the most experienced males were able to discriminate between females that had been exposed to different developmental nutritional environments (chapter 6). Among females, older, more experienced birds, but not relatively young individuals, were able to discriminate between naïve males and competent breeders (chapter 5). Most studies tend to use naïve choosers in mate preference tests (table 9.1). Whilst mating

preferences may still be observed, the use of naïve choosers that lack any pre-exposure to potential mates may explain why so few studies have found bill colour preferences. Experienced individuals may be better able to discriminate amongst the reproductive values of potential mates, especially when trait variation is low.

The other facet of mating preferences is the "choosiness" that an individual displays (*sensu* Jennions & Petrie 1997: the effort invested in mate assessment and choice). Factors such as the time and energy costs of sampling mates, the risk of predation and environmental conditions will affect choosiness (Jennions & Petrie 1997). For example, the distance travelled to sample mates by female pied flycatchers decreases in cold weather (Slagsvold *et al.* 1988). The physiological condition of an individual is also likely to influence its choosiness (Widemo & Sæther 1999). In several of my experiments, I measured the activity level of choosers (i.e. the proportion of test time that they spent affiliating with potential mates (chapter 2) or spent courting potential mates (chapters 5 and 6)), which can be considered equivalent to their choosiness. Choosiness will influence the quality of mate assessment because it determines the number of potential mates that are sampled and the thoroughness with which they are assessed (Sullivan 1994b). Thus choosiness affects how readily a preference function is expressed (Widemo & Sæther 1999). Among zebra finches, female choosiness appears to be associated with their strength of preference for high intrinsic song rate and high body mass (Forstmeier & Birkhead 2004). I observed similar associations between male choosiness and mating preferences. Relatively active males were better able to discriminate between naïve and competent females (chapter 5), females that experienced different neonatal diets (chapter 6) and between females of different body mass (chapter 2). It has been suggested that the choosiest individuals may be those of the highest quality, which would lead to assortative mating for quality (Burley 1983) in species with mutual mate choice. However, Forstmeier & Birkhead (2004) found that females did not judge the same males to be attractive, and they concluded that assortative mating for quality was not operating in their study. I found no evidence that high quality individuals were choosier; for example, choosiness was not correlated with male age or female age (chapters 2, 5 and 6). However, when intrasexual competition for mates is considered, assortative mating is likely to be fairly common in wild populations.

Zebra finch mating preferences have been examined on numerous occasions and consequently this species can tell us much about the existence of variation

between individuals and between different study populations. A perusal of the zebra finch mate choice literature highlights how problematic it can be to compare different studies, even of the same species. Experiments differ in the methodologies that they employ, in their environmental conditions (which can affect the levels of phenotypic trait variation that are observed) and in the characteristics of the animals that they use. Crucially, individuals that express mating preferences may differ in their inherited predispositions towards certain traits and in characteristics such as their previous experience and their condition. These differences can give rise to variation in mating preferences, which has important implications for the strength and shape of sexual selection on preferred traits (Jennions & Petrie 1997; Widemo & Sæther 1999).

The links between pair bond stability and the downstream effects of early nutrition

An animal's mate choice is not an invariable expression of its ideal mating preference. Animals can make initial mate assessment errors, or constraints, such as time limitations or poor chooser condition, may curb their choosiness and thereby hamper optimal mate choice (Widemo & Sæther 1999). In addition, mating preferences can be superseded by intrasexual competition for mates (Qvarnström & Forsgren 1998). One upshot of these constraints on initial mate choice, for socially monogamous species, is that pair bonds will vary in their stability (Ens *et al.* 1996). Pair bond strength may be further altered by an animal's success with an established partner and the nature of the alternative mates that are available (chapters 3 and 4). Mate choice is a dynamic process and, as such, individuals can improve upon their initial mate choice (figure 1.1).

Several of the experiments in this thesis examined the downstream effects of developmental nutrition, which is a factor that may interact with pair bond stability, as I will discuss. Zebra finches are more liable than related *Poephila* species to pair with initially non-preferred individuals (Zann 1996). As zebra finches have relatively short life spans, and reproduce opportunistically, breeding with an inferior partner is a better option than failing to pair. Thus, initial mate choice errors and acceptance of inferior mates may be common in this species. Zann (1996) tentatively concludes that mate change subsequent to pair formation (i.e. EPCs, but possibly divorce as well) may be a more viable strategy for zebra finches than investing heavily in initial mate

choice. One aspect of mate selection about which little is known is how cognitive development affects mate preferences and assessment. Developmental environment is known to affect avian song learning (Nowicki *et al.* 1998; Buchanan *et al.* 2003; Spencer *et al.* 2003; chapter 8) and appears to influence other aspects of adult cognitive performance (chapter 8). It would be interesting to examine whether early nutrition also impacts on mate perception, assessment and preferences in adulthood, as this has yet to be investigated. Poor early nutrition may conceivably impair mate assessment abilities, affect the consolidation of preference functions and alter mate-sampling strategies. In turn, this may increase errors in initial mate choice and thereby influence the subsequent stability of a pair bond.

Zebra finches appear to be able to compensate for an early nutritional deficit, at least to an extent and depending on the severity of the shortfall. Consequently, in many cases, as adults they may be indistinguishable from birds that did not experience a period of nutritional stress (Birkhead *et al.* 1999; Blount *et al.* 2003a; chapters 6-8). As a result of the importance of securing a mate early in life for this species, zebra finches may invest heavily in their own attractiveness at the expense of other traits. For example, early developmental conditions affect longevity amongst males (Birkhead *et al.* 1999), antioxidant levels in adulthood (Blount *et al.* 2003a; chapter 7), cognitive performance (chapter 8) and fecundity amongst females (Haywood & Perrins 1992; Gorman & Nager 2004). As a result, an individual may pair with an ostensibly attractive mate that experienced poor early nutrition and the impairment wrought by the nutritional deficit may be revealed only during a reproductive attempt. Pair bond stability should be reduced in such cases. It would be informative to investigate whether prenatal or neonatal nutrition exerts downstream effects on parental investment. Although developmental nutrition seems not to affect adult male bill colour (Birkhead *et al.* 1999; Blount *et al.* 2003a; chapter 6), it can have permanent, long-term effects on song syntax (Spencer *et al.* 2003; chapter 8). Song complexity is one cue that females could potentially use to inform themselves of a male's developmental background. Females appear to prefer males with complex songs (Clayton & Pröve 1989), but the importance of song syntax in relation to other sexual signals is unclear. Female zebra finches prefer to engage in EPCs with males that possess high song rates (Houtman 1992), but it may be interesting to examine whether male song syntax also influences pair bond stability.

Perspectives for future work

The results of the experiments described in this thesis suggest several avenues for future work. As a general point, as I discussed in chapter 1, the methodology of future examinations of mating preferences should be carefully considered. The design of an experiment depends on what is being investigated, but between-individual variation in preferences, the optimal test duration and the relevance of test affiliation behaviour to actual mating patterns (and therefore sexual selection) should be borne in mind.

I found some evidence that breeding performance affects pair bond stability in captive zebra finches (chapter 4). It would be useful to exclude an alternative explanation for these results. Namely, unsuccessful pairs may have spent less time incubating than successful pairs over the course of the breeding attempts. Consequently, they may have become more familiar than successful pairs with their neighbours. Cages could be set up so that birds were required to actively search for their neighbours in order to assess them. The amount of time spent assessing neighbouring pairs could then be recorded. If these values were not different between successful and unsuccessful pairs then the alternative explanation could be excluded.

Future investigations into avian pair bond stability may benefit from recording baseline costs and benefits of pair bond duration and divorce. For example, we know that individual age and experience have very limited effects on reproductive performance in the zebra finch (Zann 1994; Williams & Christians 2003). However, it is not known whether breeding success increases with pair bond duration. Knowledge of the relationship between parental investment (and breeding performance) and morphological traits that have been measured in a study species would provide an estimation of the potential benefits of divorce for a better option. Thus, an understanding of the baseline costs and benefits of divorce would allow "divorce thresholds" to be estimated and predictions to be made. Furthermore, measurements of initial mating preferences for an established partner prior to a breeding attempt would then allow a comparison with post-breeding preferences. This may provide a more accurate indication of the effect of breeding performance on pair bond stability.

Several studies have examined how mating preferences vary with the age and experience of potential mates (Burley & Moran 1979; Schubert et al. 1989; chapter 5). However, the effects of a mating option's age and experience on mating preferences have yet to be satisfactorily separated and this would be a useful experiment to

conduct. In zebra finches specifically, it remains unclear why competent mates are preferred over naïve mates (Schubert *et al.* 1989; chapter 5). How do choosers discriminate between competent and naïve birds? Schubert *et al.* (1989) proposed that courtship displays and co-ordination may improve with age and, in order for this to be substantiated, detailed observations of courtship at different ages would be useful.

Future studies that investigate the downstream effects of early nutritional restriction should consider examining the negative influence of both the nutritional stress itself and any period of growth compensation that occurs. It is now clear that both nutritional stress and compensatory growth can impair adult fitness and it is important to separate their effects (Metcalfe & Monaghan 2001). For example, I found that developmental conditions influenced adult antioxidant defences, but the relative importance of slow neonatal growth and subsequent compensatory growth was uncertain. The negative effects of developmental stress may be shrouded to some extent by benign laboratory conditions in some studies. Therefore, future studies should consider presenting challenges, for example, during windows that are available for compensatory growth (e.g. immune challenges; increased workloads). More challenging experimental conditions may also increase the benefits of choosing a genuinely "high-quality mate", thereby increasing sexual selection within captive populations in the long term. Amongst birds that received experimental neonatal diets, post-fledging mortality was much higher in females than in males (chapter 7). It would be instructive to examine the links between early diet and adult mortality further, particularly sex-differences in responses to specific dietary components. The connection between compensatory growth and adult cognitive ability (chapter 8) leaves much scope for future studies. Through what mechanism does accelerated growth rate impinge on cognitive performance? Are certain parts of the brain differentially affected? What implications do these findings have for adult fitness?

References

Aihie Sayer, A. & Cooper, C. 2002. Early diet and growth: impact on ageing. *Proceedings of the Nutritional Society*, **61**, 79-85.

Aihie Sayer, A., Dunn, R., Langley-Evans, S. & Cooper, C. 2001. Prenatal exposure to a maternal low protein diet shortens life span in rats. *Gerontology*, **47**, 9-14.

Airey, D. C., Castillo-Juarez, H., Casella, G., Pollak, E. J. & DeVoogd, T. J. 2000. Variation in the volume of zebra finch song control nuclei is heritable: developmental and evolutionary implications. *Proceedings of the Royal Society, London, Series B*, **267**, 2099-2104.

Alatalo, R. V., Höglund, J. & Lundberg, A. 1988. Patterns of variation in tail ornament size in birds. *Biological Journal of the Linnean Society*, **34**, 363-374.

Alisauskas, R. T. & Ankney, C. D. 1990. Body size and fecundity in lesser snow geese. *Auk*, **107**, 440-443.

Alvarez-Buylla, A., Ling, C.-Y. & Nottebohm, F. 1992. High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries. *Journal of Neurobiology*, **23**, 396-406.

Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology and Evolution*, **15**, 149-155.

Amundsen, T., Forsgren, E. & Hansen, L. T. T. 1997. On the function of female ornaments: male bluethroats prefer colourful females. *Proceedings of the Royal Society, London, Series B*, **264**, 1579-1586.

Andersson, M. B. 1994. *Sexual Selection*. Princetown University Press.

Arcese, P. & Smith, J. N. M. 1985. Phenotypic correlates and ecological consequences of dominance in song sparrows. *Journal of Animal Ecology*, **54**, 817-830.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. *Quarterly Review of Biology*, **72**, 149-177.

Baeyens, G. 1981. Functional aspects of serial monogamy: the magpie pair-bond in relation to its territorial system. *Ardea*, **69**, 145-166.

Balzer, A. L. & Williams, T. D. 1998. Do female zebra finches vary primary reproductive effort in relation to mate attractiveness? *Behaviour*, **135**, 297-309.

Barbraud, C., Weimerskirch, H., Robertson, G. & Jouventin, P. 1999. Size-related life history traits: insights from a study of snow petrels (*Pagodroma nivea*). *Journal of Animal Ecology*, **68**, 1179-1192.

Barker, D. J. P. *et al.* 1993. Fetal nutrition and cardiovascular disease in adult life. *The Lancet*, **341**, 938-941.

Basolo, A. L. 2004. Variation between and within the sexes in body size preferences. *Animal Behaviour*, **68**, 75-82.

Bateson, P. 1983. *Mate choice* (ed. Bateson, P.). Cambridge University Press, Cambridge.

Bateson, P. *et al.* 2004. Developmental plasticity and human health. *Nature*, **430**, 419-421.

Beckman, K. B. & Ames, B. N. 1998. The free radical theory of ageing matures. *Physiological Reviews*, **78**, 547-581.

Belthoff, J. R. & Gauthreaux, Jr, S. A. 1991. Aggression and dominance in house finches. *The Condor*, **93**, 1010-1013.

Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. & Maier, E. J. 1996. Ultraviolet vision and mate choice in zebra finches. *Nature*, **380**, 433-435.

Birkhead, T. R., Burke, T., Zann, R., Hunter, F. M. & Krupa, A. P. 1990. Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behavioural Ecology and Sociobiology*, **27**, 315-324.

Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1998. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. *Behavioral Ecology and Sociobiology*, **44**, 179-191.

Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. *Proceedings of the Royal Society, London, Series B*, **266**, 385-390.

Birkhead, T. R., Hunter, F. M. & Pellat, J. E. 1989. Sperm competition in the zebra finch *Taeniopygia guttata*. *Animal Behaviour*, **38**, 935-950.

Birkhead, T. R. & Møller, A. P. 1992. *Sperm competition in birds*. Academic Press, London.

Bisazza, A., Marconato, A. & Marin, G. 1989. Male competition and female choice in *Padogobius martensi* (Pisces, Gobiidae). *Animal Behaviour*, **38**, 406-413.

Black, J. M. 1996. Introduction: pair bonds and partnerships. In *Partnerships in birds: the study of monogamy* (ed. Black, J. M.), pp. 3-20. Oxford University Press.

Blanckenhorn, U. 2000. The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, **75**, 385-407.

Blondel, J., Maistre, M., Perret, P., Hurtrez-Boussès, S. & Lambrechts, M. M. 1998. Is the small clutch size of a Corsican blue tit population optimal? *Oecologia*, **117**, 80-89.

Blondel, J., Perret, P. & Galan, M-J. 2000. High divorce rates in Corsican blue tits: how to choose a better option in a harsh environment. *Oikos*, **89**, 451-460.

Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., Devevey, G. L. & Monaghan, P. 2003a. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proceedings of the Royal Society, London, Series B*, **270**, 1691-1696.

Blount, J. D., Metcalfe, N. B., Birkhead, T. R. & Surai, P. F. 2003b. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science*, **300**, 125-127.

Blums, P., Clark, R. G. & Mednis, A. 2002. Patterns of reproductive effort and success in birds: path analyses of long-term data from European ducks. *Journal of Animal Ecology*, **71**, 280-295.

Boag, P. T. 1987. Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). *The Auk*, **104**, 155-166.

Borras, A., Pascual, J. & Senar, J. C. 2000. What do different bill measures measure and what is the best method to use in granivorous birds? *Journal of Field Ornithology*, **71**, 606-611.

Brooks, R. & Kemp, D. J. 2001. Can older males deliver the good genes? *Trends in Ecology and Evolution*, **16**, 308-313.

Bryant, D. M. & Newton, A. V. 1994. Metabolic costs of dominance in dippers, *Cinclus cinclus*. *Animal Behaviour*, **48**, 447-455.

Buchanan, K. L. & Catchpole, C. K. 2000. Song as an indicator of male parental effort in the sedge warbler. *Proceedings of the Royal Society, London, Series B*, **267**, 321-326.

Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A.R. & Catchpole, C. K. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proceedings of the Royal Society, London, Series B*, **271**, 2381-2386.

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R. & Catchpole, C. K. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society, London, Series B*, **270**, 1149-1156.

Burley, N. 1977. Parental investment, mate choice, and mate quality. *Proceedings of the National Academy of Science,* USA, **74**, 3476-3479.

Burley, N. 1983. The meaning of assortative mating. *Ethology and Sociobiology*, **4**, 191-203.

Burley, N. 1985. Leg-band colour and mortality patterns of captive breeding populations of zebra finches. *Auk*, **102**, 647-651.

Burley, N. & Coopersmith, C. B. 1987. Bill colour preferences of zebra finches. *Ethology*, **76**, 133-151.

Burley, N. T. & Johnson, K. 2002. The evolution of parental care. *Philosophical Transactions of the Royal Society, London, Series B*, **357**, 241-250.

Burley, N., Krantzberg, G. & Radman, P. 1982. Influence of colour-banding on the preferences of zebra finches. *Animal Behaviour*, **30**, 444-455.

Burley, N. & Moran, N. 1979. The significance of age and reproductive experience in the mate preferences of feral pigeons, *Columba livia*. *Animal Behaviour*, **27**, 686-698.

Butterfield, P. A. 1970. The pair bond in the zebra finch. In: *Social behaviour in birds and mammals* (Ed: Crook, J. H.), pp. 249-278. Academic Press, London.

Campbell, T. W. 1995. *Avian haematology and cytology*. Iowa State University Press.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews*, **78**, 575-595.

Carrascal, L. M., Senar, J. C., Mozetich, I., Uribe, F. & Domenech, J. 1998. Interactions among environmental stress, body condition, nutritional status, and dominance in great tits. *Auk*, **115**, 727-738.

Caryl, P. G. 1975. Aggressive behaviour in the zebra finch *Taeniopygia guttata*. I. Fighting provoked by male and female social partners. *Behaviour*, **52**, 226-252.

Catchpole, C. K. 1987. Bird song, sexual selection and female choice. *Trends in* Ecology and Evolution, **2**, 94-97.

Catry, P., Ratcliffe, N. & Furness, R. W. 1997. Partnerships and mechanisms of divorce in the great skua. *Animal Behaviour*, **54**, 1475-1482.

Cézilly, F. & Nager, R. G. 1995. Comparative evidence for a positive association between divorce and extra-pair paternity in birds. *Proceedings of the Royal Society, London, SeriesB*, **262**, 7-12.

Cézilly, F., Préault, M., Dubois, F., Faivre, B. & Patris, B. 2000. Pair-bonding in birds and the active role of females: a critical review of the empirical evidence. *Behavioural Processes*, **51**, 83-92.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends in Ecology and Evolution*, **18**, 41-47.

Choudhury, S. 1995. Divorce in birds: a review of the hypotheses. *Animal Behaviour*, **50**, 413-429.

Clayton, N. S. 1990. Mate choice and pair formation in Timor and Australian mainland zebra finches. *Animal Behaviour*, **39**, 474-480.

Clayton, N. & Pröve, E. 1989. Song discrimination in female Zebra Finches and Bengalese Finches. *Animal Behaviour*, **38**, 352-354.

Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society, London, Series B*, **235**, 339-372.

Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature*, **351**, 58-60.

Collins, S. A. 1995. The effect of recent experience on female choice in zebra finches. *Animal Behaviour*, **49**, 479-486.

Collins, S. A., Hubbard, C. & Houtman, A. M. 1994. Female mate choice in the zebra finch - the effect of male beak colour and male song. *Behavioral Ecology and Sociobiology*, **35**, 21-25.

Collins, S. A. & ten Cate, C. 1996. Does beak colour affect female preference in zebra finches? *Animal Behaviour*, **52**, 105-112.

Colwell, M. A. & Oring, L. W. 1989. Extra-pair mating in the spotted sandpiper: a female mate acquisition tactic. *Animal Behaviour*, **38**, 675-684.

Comfort, A. 1979. *The biology of senescence, third edition*. Churchill Livingstone, Edinburgh.

Côte, I. M. & Hunte, W. 1989. Male and female mate choice in the redlip blenny: why bigger is better. *Animal Behaviour*, **38**, 78-88.

Coulson, J. C. & Thomas, C. S. 1983. Mate choice in the kittiwake gull. In: *Mate choice* (ed. Bateson, P.), pp. 361-376. Cambridge University Press, Cambridge.

Coyle, J. T. & Puttfarcken, P. 1993. Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, **262**, 689-695.

Crawley, M. J. 2002. *Statistical computing: an introduction to data analysis using S-Plus*. John Wiley & Sons Ltd, Chichester.

Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution*, **16**, 491-497.

Cuthill, I. C., Hunt, S., Cleary, C. Clark, C. 1997. Colour bands, dominance, and body mass regulation in male zebra finches (*Taeniopygia guttata*). *Proceedings of the Royal Society, London, Series B*, **264**, 1093-1099.

Davies, N. B. 1989. Sexual conflict and the polygyny threshold model. *Animal Behaviour*, **38**, 226-234.

Dearborn, D. C. & Wiley, R. H. 1993. Prior residence has a gradual influence on dominance in captive white-throated sparrows. *Animal Behaviour*, **46**, 39-46.

de Kogel, C. H. 1997. Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. *Journal of Animal Ecology*, **66**, 167-178.

de Kogel, C. H. & Prijs, H. J. 1996. Effects of brood size manipulations on sexual attractiveness of offspring in the zebra finch. *Animal Behaviour*, **51**, 699-708.

Desai, M. & Hales, C. N. 1997. Role of fetal and infant growth in programming metabolism in later life. *Biological Reviews*, **72**, 329-348.

Desrochers, A. & Magrath, R. D. 1996. Divorce in the European Blackbird: seeking greener pastures? In: *Partnerships in birds: the study of monogamy* (Ed. Black, J. M.), pp. 177-191. Oxford University Press, Oxford.

DeVries, A. C., DeVries, M. B., Taymans, S. & Carter, C. S. 1995. Modulation of pair bonding in female prairie voles (*Microtus ochrogaster*) by corticosterone. *Proceedings of the National Academy of Science, USA*, **92**, 7744-7748.

Dhondt, A. A. 2002. Changing mates. *Trends in Ecology and Evolution*, **17**, 55-56.

Dhondt, A. A. & Adriaensen, F. 1994. Causes and effects of divorce in the blue tit *Parus caerulus*. *Journal of Animal Ecology*, **63**, 979-987.

Dillon, J. L. L. D. & Wu, E. 1990. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. *American Journal of Physiology*, **258**, R918-R923.

Dubois, F. & Cézilly, F. 2002. Breeding success and mate retention in birds: a meta-analysis. *Behavioral Ecology and Sociobiology*, **52**, 357-364.

Ekman, J. B. & Askenmo, C. E. H. 1984. Social rank and habitat use in willow tit groups. *Animal Behaviour*, **32**, 508-514.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215-223.

Endler, J. A. & Basolo, A. 1998. Sensory ecology, receiver biases and sexual selection. *Trends in Ecology and Evolution*, **13**, 415-420.

Ens, B. J., Choudhury, S. & Black, J. M. 1996. Mate fidelity and divorce in monogamous birds. In: *Partnerships in birds: the study of monogamy* (ed. Black, J. M.), pp. 344-395. Oxford University Press, Oxford.

Ens, B. J., Safriel, U. N. & Harris, M. P. 1993. Divorce in the long-lived and monogamous oystercatcher, *Haematopus ostralegus*: incompatibility or choosing the better option? *Animal Behaviour*, **45**, 1199-1217.

Eriksson, J. G., Forsén, T., Tuomilehto, J., Winter, P. D., Osmond, C. & Barker, D. J. P. 1999. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *British Medical Journal*, **318**, 427-431.

Eshel, I., Volovik, I. & Sansone, E. 2000. On Fisher-Zahavi's handicapped sexy son.

Evolutionary Ecology Research, **2**, 509-523.

Etches, R. J. 1996. *Reproduction in poultry*. CAB International, Oxford.

Fernstrom, J. D. 2000. Can nutrient supplements modify brain function? *American* Journal of Clinical Nutrition, **71**, 1669S-1673S.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford University Press, Oxford.

Fleming, J. E, Reveillaud, I. & Niedzwiecki, A. 1992. Role of oxidative stress in *Drosophila* aging. *Mutation Research*, **275**, 267-279.

Forsén, T., Eriksson, J. G., Tuomilehto, J., Osmond, C. & Barker, D. J. P. 1999. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. *British Medical Journal*, **319**, 1403-1407.

Forsgren, E. 1997. Female sand gobies prefer good fathers over dominant males. Proceedings of the Royal Society, London, Series B, **264**, 1283-1286.

Forslund, P. & Pärt, T. 1995. Age and reproduction in birds – hypotheses and tests. *Trends in Ecology and Evolution*, **10**, 374-378.

Forstmeier, W. 2004. Female resistance to male seduction in zebra finches. *Animal Behaviour*, **68**, 1005-1015.

Forstmeier, W. & Birkhead, T. R. 2004. Repeatability of mate choice in the zebra finch: consistency within and between females. *Animal Behaviour*, **68**, 1017-1028.

Gebhardt-Henrich, S. & Richner, H. 1998. Cause of growth variation and its consequences for fitness. In: *Avian growth and development: evolution within the altricial-precocial spectrum* (ed. Starck, J. M. & Ricklefs, R. E.), pp. 324-339. Oxford University Press.

Gil, D., Naguib, M., Riebel, K., Rutstein, A. & Gahr, M. 2004. Developmental stress and song learning in zebra finches. Abstract, *European Conference on Behavioural* Biology 2004, Groningen.

Gillette, J. R., Jaeger, R. G. & Peterson, M. G. 2000. Social monogamy in a territorial salamander. *Animal Behaviour*, **59**, 1241-1250.

Gorman, H. E. & Nager, R. G. 2003. State-dependent incubation behaviour in the zebra finch. *Animal Behaviour*, **65**, 745-754.

Gorman, H. E. & Nager, R. G. 2004. Prenatal developmental conditions have long-

term effects on offspring fecundity. *Proceedings of the Royal Society, London, Series B*, **271**, 1923-1928.

Gosler, A. G. 1996. Environmental and social determinants of winter fat storage in the great tit *Parus major*. *Journal of Animal Ecology*, **65**, 1-17.

Gowaty, P. A. 1996. Battles of the sexes and origins of monogamy. In *Partnerships in birds: the study of monogamy* (ed. Black, J. M.), pp. 21-52. Oxford University Press.

Gustafsson, L., Qvarnström, A. & Sheldon, B. C. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature*, **375**, 311-313.

Gwynne, D. T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. *Science*, **213**, 779-780.

Hales, C. N. & Ozanne, S. E. 2003. The dangerous road of catch-up growth. *Journal of* Physiology, **547**, 5-10.

Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384-387.

Hannon, S. & Martin, K. 1996. Mate fidelity and divorce in ptarmigan: polygyny avoidance on the tundra. In: *Partnerships in birds: the study of monogamy* (ed. Black, J. M.), pp. 192-210. Oxford University Press, Oxford.

Hansen, T. F. & Price, D. K. 1995. Good genes and old age: Do old mates provide superior genes? *Journal of Evolutionary Biology*, **8**, 759-778.

Hauser, M. D. 1996. *The evolution of communication*. MIT Press.

Hawkins, P., Morton, D. B., Cameron, D., Cuthill, I., Francis, R., Freire, R., Gosler, A., Healy, S., Hudson, A., Inglis, I., Kirkwood, J., Lawton, M., Monaghan, P., Sherwin, C. & Townsend, P. 2001. Laboratory birds: refinements in husbandry and procedures. *Laboratory animals*, **35**, S1-S163.

Hay, J. M., Evans, P. R., Ward, R. M. & Hamer, K. C. 2004. Poor nutritional condition as a consequence of high dominance status in the coal tit *Parus ater*. *Ibis*, **146**, 103-107.

Haywood, S. & Perrins, C. M. 1992. Is clutch size in birds affected by environmental conditions during growth? *Proceedings of the Royal Society, London, Series B*, **249**,

195-197.

Heg, D., Ens, B., Burke, R. T., Jenkins, L. & Kruijt, J. P. 1993. Why does the typically monogamous oystercatcher (*Haematopus ostralegus*) engage in extra-pair copulations? *Behaviour*, **126**, 247-288.

Hegner, R. E. 1985. Dominance and anti-predator behaviour in blue tits (*Parus caeruleus*). *Animal Behaviour*, **33**, 762-768.

Heisler, I. L., Andersson, M. B., Arnold, S. T., Boake, C. R., Borgia, G., Hausfater, G., Kirkpatrick, M., Lande, R., Maynard Smith, J., O'Donald, P., Thornhill, R. & Weissing, F. J. 1987. The evolution of mating preferences and sexually selected traits. In: *Sexual selection: testing the alternatives* (ed. Bradbury, J. W. & Andersson, M. B.), pp. 96-118. John Wiley & Sons, New York.

Henry, C. J. K. & Ulijaszek, S. J. 1996. *Long-term consequences of early environment.* Cambridge University Press.

Heywood, J. S. 1989. Sexual selection by the handicap mechanism. *Evolution*, **43**, 1387-1397.

Hoelzer, G. A. 1989. The good parent process of sexual selection. *Animal Behaviour*, **38**, 1067-1078.

Hogstad, O. 1987. It is expensive to be dominant. *Auk*, **104**, 333-336.

Hogstad, O. 1988. Rank-related resource access in winter flocks of willow tit *Parus montanus*. *Ornis Scandinavica*, **19**, 169-174.

Holland, B. & Rice, W. R. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, **52**, 1-7.

Houtman, A. M. 1990. Sexual selection in the zebra finch, *Poephila guttata*. Dphil thesis, University of Oxford.

Houtman, A. M. 1992. Female zebra finches choose extra-pair copulations with genetically attractive males. *Proceedings of the Royal Society, London, Series B*, **249**, 3-6.

Huang, D., Ou, B. & Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, **53**, 1841-1856.

Hunt, S., Cuthill, I. C., Bennett, A. T. D., Church, S. C. & Partridge, J. C. 2001. Is the ultraviolet waveband a special communication channel in avian mate choice?

Journal of Experimental Biology, **204**, 2499-2507.

Immelmann, K. 1965. Australian Finches in bush and aviary. Angus and Robertson, Sydney.

Jennings, B. J., Ozanne, S. E., Dorling, M. W. & Hales, C. N. 1999. Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS Letters*, **448**, 4-8.

Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological reviews*, **72**, 283-327.

Johnson, J. B. & Omland, K. S. 2004. Model selection in ecology and evolution. *Trends in ecology and evolution*, **19**, 101-108.

Johnson, K. 1988. Sexual selection in pinyon jays II: male choice and female-female competition. *Animal Behaviour*, **36**, 1048-1053.

Johnstone, R. A., Reynolds, J. D. & Deutsch, J. C. 1996. Mutual mate choice and sex differences in choosiness. *Evolution*, **50**, 1382-1391.

Jones, I. L. & Hunter, F. M. 1993. Mutual sexual selection in a monogamous seabird. *Nature*, **362**, 238-239.

Jones, I. L. & Montgomerie, R. 1991. Mating and remating of least auklets (*Aethia **pusilla***) relative to ornamental traits. *Behavioral Ecology,* **2,** 249-257.

Jones, K. M., Monaghan, P. & Nager, R. G. 2001. Male mate choice and female fecundity in zebra finches. *Animal Behaviour*, **62**, 1021-1026.

Jones, T. M., Balmford, A. & Quinnell, R. J. 2000. Adaptive female choice for middle-aged mates in a lekking sandfly. *Proceedings of the Royal Society, London, Series B*, **267**, 681-686.

Kikkawa, J. 1980. Winter survival in relation to dominance classes among silvereyes *Zosterops lateralis chlorocephala* of Heron Island, Great Barrier Reef. *Ibis*, **122**, 437-446.

Kim, J. J. & Yoon, K. S. 1998. Stress: metaplastic effects in the hippocampus. *Trends* in Neurosciences, **21**, 505-509.

Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature*, **350**, 33-38.

Kokko, H. 1997. Evolutionary stable strategies of age-dependent sexual advertisement. *Behavioral Ecology and Sociobiology*, **41**, 99-107.

Kokko, H. 1998. Good genes, old age and life-history trade-offs. *Evolutionary Ecology*, **12**, 739-750.

Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. 2003. The evolution of mate choice and mating biases. *Proceedings of the Royal Society, London, Series B*, **270**, 653-664.

Kokko, H., Brooks, R., McNamara, J. M. & Houston, A. I. 2002. The sexual selection continuum. *Proceedings of the Royal Society, London, Series B*, **269**, 1331-1340.

Kokko, H. & Johnstone, R. A. 2002. Why is mutual mate choice not the norm? Parental investment, operational sex ratios, and sex roles. *Philosophical Transactions of the Royal Society, London, Series B*, **357**, 319-330.

Kokko, H. & Lindström, J. 1996. Evolution of female preference for old mates. *Proceedings of the Royal Society, London, Series B*, **263**, 1533-1538.

Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. *Ecology Letters*, **4**, 159-165.

Kraak, S. B. M. & Bakker, T. C. M. 1998. Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. *Animal Behaviour*, **56**, 859-866.

Lande, R. 1980. Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution*, **34**, 292-305.

Lifjeld, J. T. & Slagsvold, T. 1988. Mate fidelity of renesting pied flycatchers *Ficedula hypoleuca* in relation to characteristics of the pair mates. *Behavioral Ecology and Sociobiology*, **22**, 117-123.

Lindén, M. 1991. Divorce in great tits – chance or choice? An experimental approach. *The American Naturalist*, **138**, 1039-1048.

Lindstrom, A. & Piersma, T. 1993. Mass changes in migrating birds – the evidence for fat and protein storage re-examined. *Ibis*, **135**, 70-78.

Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14**, 343-348.

Lucas, A. 1998. Programming by early nutrition: an experimental approach. *Journal*

of Nutrition, **128**, 401S-406S.

Lucas, A., Baker, B. A., Desai, M. & Hales, C. N. 1996. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. *British Journal of Nutrition*, **76**, 605-612.

Lucas, A., Morley, R. & Cole, T. J. 1998. Randomised trial of early diet in preterm babies and later intelligence quotient. *British Medical Journal*, **317**, 1481-1487.

Lucas, A., Morley, R., Cole, T. J., Lister, G. & Leeson-Payne, C. 1992. Breast milk and subsequent intelligence quotient in children born preterm. *The Lancet*, **339**, 261-264.

McNamara, J. M. & Forslund, P. 1996. Divorce rates in birds: predictions from an optimisation model. *American Naturalist*, **147**, 609-640.

McNamara, J. M., Forslund, P. & Lang, A. 1999. An ESS model for divorce strategies in birds. *Philosophical Transactions of the Royal Society, London, Series B*, **354**, 223-236.

McNamara, J. M., Houston, A. I., dos Santos, M. M., Kokko, H. & Brooks, R. 2003. Quantifying male attractiveness. *Proceedings of the Royal Society, London, Series B*, **270**, 1925-1932.

Manning, J. T. 1985. Choosy females and correlates of male age. *Journal of Theoretical Biology*, **116**, 349-354.

Masoro, E. J. & Austad, S. N. 2001. *Handbook of the biology of aging, 5th edition.* Academic Press, London.

Matthysen, E. 1990. Behavioral and ecological correlates of territory quality in the Eurasian nuthatch (*Sitta europaea*). *The Auk*, **107**, 86-95.

Mays Jr, H. L. & Hill, G. E. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology and Evolution*, **19**, 554-559.

Metcalfe, N. B. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution*, **16**, 254-260.

Metcalfe, N. B. & Monaghan, P. 2003. Growth versus lifespan: perspectives from evolutionary ecology. *Experimental Gerontology*, **38**, 935-940.

Møller, A. P. & Thornhill, R. 1998. Bilateral symmetry and sexual selection: a meta-analysis. *American Naturalist*, **151**, 174-192.

Monaghan, P., Metcalfe, N. B. & Houston, D. C. 1996. Male finches selectively pair with fecund females. *Proceedings of the Royal Society, London, Series B*, **263**, 1183-1186.

Morris, D. 1954. The reproductive behaviour of the zebra finch (Poephila guttata), with special reference to pseudofemale behaviour and displacement activities. *Behaviour*, **6**, 271-322.

Motro, U. 1982. The courtship handicap: phenotypic effect. *Journal of Theoretical Biology*, **97**, 319-324.

Newton, I. 1989. *Lifetime Reproduction in birds*. Academic Press, London.

Nowicki, S., Hasselquist, D., Bensch, S. & Peters, S. 2000. Nestling growth and song repetoire size in great reed warblers: evidence for song learning as an indicator mechanism in mate choice. *Proceddings of the Royal Society, London, Series B*, **267**, 2419-2424.

Nowicki, S., Peters, S. & Podos, J. 1998. Song learning, early nutrition and sexual selection in songbirds. *American Zoologist*, **38**, 179-190.

Nowicki, S., Searcy, W. A. & Peters, S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". *Journal of Comparative Physiology A*, **188**, 1003-1014.

Nutting, D. F., Kumar, N. S., Siddiqi, S. A. & Mansbach, C. M. 2002. Nutrient absorption. Current Opinion in Gasteroenterology, **18**, 168-175.

Oberweger, K. & Goller, F. 2001. The metabolic cost of birdsong production. *Journal of Experimental Biology*, **204**, 3379-3388.

O'Connor, R. J. 1984. *The growth and development of birds*. Wiley & Sons, New York.

Ohlsson, T., Smith, H. G., Råberg, L. & Hasselquist, D. 2002. Pheasant sexual ornaments reflect nutritional conditions during early growth. *Proceedings of the Royal Society, London, Series B*, **269**, 21-27.

Olsson, M. 1993. Male preference for large females and assortative mating for body size in the sand lizard (*Lacerta agilis*). *Behavioral Ecology and Sociobiology*, **32**, 337-341.

Orell, M., Rytkönen, S. & Koivula, K. 1994. Causes of divorce in the monogamous

willow tit, *Parus montanus*, and consequences for reproductive success. *Animal Behaviour*, **48**, 1143-1154.

Otter, K. & Ratcliffe, L. 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. *Proceedings of the Royal Society, London, Series B*, **263**, 351-354.

Owen, M., Black, J. M. & Liber, H. 1988. Pair bond duration and the timing of its formation in barnacle geese. In: *Wildfowl in Winter* (ed. by Weller, M. W.), pp. 23-38. Minneapolis, University of Minnesota Press.

Owens, I. P. & Thompson, D. B. A. 1994. Sex differences, sex ratios and sex roles. *Proceedings of the Royal Society, London, Series B*, **258**, 93-99.

Ozanne, S. E. & Hales, C. N. 2002. Early programming of glucose-insulin metabolism. Trends in Endocrinology and Metabolism, **13**, 368-373.

Ozanne, S. E. & Hales, C. N. 2004. Catch-up growth and obesity in male mice. *Nature*, **427**, 411-412.

Parker, G. A. 1983. Mate quality and mating decisions. In: *Mate choice* (ed. Bateson. P.), pp. 141-166. Cambridge University Press, Cambridge.

Peters, A., Denk, A. G., Delhey, K. & Kempenaers, B. 2004. Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *Journal of Evolutionary Biology*, **17**, 1111-1120.

Peters, W. D. & Grubb Jr, T. C. 1983. An experimental analysis of sex-specific foraging in the downy woodpecker, *Picoides pubescens*. *Ecology*, **64**, 1437-1443.

Piersma, T. & Davidson, N. C. 1991. Confusions of mass and size. *Auk*, **108**, 441-443.

Piper, W. H. & Wiley, R. H. 1990. The relationship between social dominance, subcutaneous fat, and annual survival in wintering white-throated sparrows (*Zonotrichia albicollis*). *Behavioral Ecology and Sociobiology*, **26**, 201-208.

Pitts, G. C. 1986. Cellular aspects of growth and catch-up growth in the rat: a reevaluation. *Growth*, **50**, 419-436.

Price, D. K. 1996. Sexual selection, selection load and quantitative genetics of zebra finch bill colour. *Proceedings of the Royal Society, London, Series B*, **263**, 217-221.

Price, D. K. & Burley, N. T. 1993. Constraints on the evolution of attractive traits: genetic (co)variance of zebra finch bill colour. *Heredity*, **71**, 405-412.

Price, T., Schluter, D. & Heckman, N. E. 1993. Sexual selection when the female directly benefits. *Biological Journal of the Linnean Society*, **48**, 187-211.

Proulx, S. R., Day, T. & Rowe, L. 2002. Older males signal more reliably. Proceedings of the Royal Society, London, Series B, **269**, 2291-2299.

Qvarnström, A. 1997. Experimentally increased badge size increases male competition and reduces male parental care in the collared flycatcher. *Proceedings of the Royal Society, London, Series B*, **264**, 1225-1231.

Qvarnström, A. & Forsgren, E. 1998. Should females prefer dominant males? *Trends in Ecology and Evolution*, **13**, 498-501.

Ramsay, S. M., Otter, K. A., Mennill, D. J., Ratcliffe, L. M. & Boag, P. T. 2000. Divorce and extrapair mating in female black-capped chickadees (*Parus Atricapillus*): separate strategies with a common target. *Behavioral Ecology and Sociobiology*, **49**, 18-23.

Ravelli, A. C. J., van der Meulen, J. H. P., Michels, R. P. J., Osmond, C., Barker, D. J. P., Hales, C. N. & Bleker, O. P. 1998. Glucose tolerance in adults after prenatal exposure to famine. *The Lancet*, **351**, 173-177.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, **26**, 1231-1237.

Reid, J. M. *et al.* 2004. Song repetoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. *Animal Behaviour*, **68**, 1055-1063.

Reid, M. L. & Weatherhead, P. J. 1990. Mate-choice criteria of Ipswich sparrows: the importance of variability. *Animal Behaviour*, **40**, 538-544.

Remage-Healey, L., Adkins-Regan, E. & Romero, L. M. 2003. Behavioral and adrenocortical responses to mate separation and reunion in the zebra finch. *Hormones and Behavior*, **43**, 108-114.

Reynolds, J. D. 1996. Animal breeding systems. *Trends in Ecology and Evolution*, **11**, 68-72.

Reynolds, J. D., & Gross, M. R. 1990. Costs and benefits of female mate choice: is

there a lek paradox? *The American Naturalist*, **136**, 230-243.

Richner, H. 1992. The effect of extra food on fitness in breeding carrion crows. *Ecology*, **73**, 330-335.

Ricklefs, R. E. 1983. Avian postnatal development. In: *Avian Biology* (Eds: Farner, D. S., King, J. R. & Parkes, K. C.), 1-83, Academic Press, New York.

Rollo, C. D. 2002. Growth negatively impacts the life span of mammals. *Evolution and Development*, **4**, 55-61.

Rose, R. C. & Wilson, J. X. 1997. Ascorbate membrane transport properties. In: *Vitamin C in health and disease* (ed. Packer, L. & Fuchs, J.), 143-162. Marcel Dekker, New York.

Ryan, M. J. 1997. Sexual selection and mate choice. In: *Behavioural ecology: an evolutionary approach, fourth edition* (ed. Krebs, J. R. & Davies, N. B.). Blackwell Science Ltd, Oxford.

Sæther, B., Lorentsen, S., Tveraa, T., Andersen, R. & Pedersen, H. C. 1997. Size-dependent variation in reproductive success of a long-lived seabird, the antarctic petrel (*Thalassoica antarctica*). *Auk*, **114**, 333-340.

Schew, W. A. & Ricklefs, R. E. 1998. Developmental Plasticity. In: *Avian growth and development: evolution within the altricial-precocial spectrum* (ed. Starck J. M. & Ricklefs, R. E.), pp. 288-304. Oxford University Press.

Schubert, C. A., Ratcliffe, L. M. & Boag, P. T. 1989. A test of inbreeding avoidance in the zebra finch. *Ethology*, **82**, 265-274.

Scrimshaw, N. S. 1998. Malnutrition, brain development, learning, and behavior. *Nutrition Research*, **18**, 351-379.

Searcy, W. A. & Andersson, M. 1986. Sexual selection and the evolution of song. *Annual Review of Ecology and Systematics*, **17**, 507-533.

Searcy, W. A., Peters, S. & Nowicki, S. 2004. Effects of early nutrition on growth rate and adult size in song sparrows *Melospiza melodia*. *Journal of Avian Biology*, **35**, 269-279.

Selman, R. G. & Houston, D. C. 1996. The effect of prebreeding diet on reproductive output in zebra finches. *Proceedings of the Royal Society, London, Series B*, **263**, 1585-1588.

Senar, J. C., Polo, V., Uribe, F. & Camerino, M. 2000. Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. *Animal Behaviour*, **59**, 103-110.

Silcox, A. P. & Evans, S. M. 1982. Factors affecting the formation and maintenance of pair bonds in the zebra finch, *Taeniopygia guttata*. *Animal Behaviour*, 30, 1237-1243.

Singhal, A., Cole, T. J., Fewtrell, M. & Lucas, A. 2004. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *The Lancet*, **363**, 1571-1578.

Singhal, A. & Lucas, A. 2004. Early origins of cardiovascular disease: is there a unifying hypothesis? *The Lancet*, **363**, 1642-1645.

Slagsvold, T., Lifjeld, T. J., Stenmark, G. & Breiehagen, T. 1988. On the cost of searching for a mate in female pied flycatchers *Ficedula hypoleuca*. *Animal Behaviour*, 36, 433-442.

Slater, J. J. B., Eales, L. A. & Clayton, N. S. 1988. Song learning in zebra finches (*Taeniopygia guttata*): progress and prospects. *Advances in the Study of Behaviour*, **18**, 1-34.

Smart, J. 1986. Undernutrition, learning and memory: review of experimental studies. In: *Proceedings of XII international congress of nutrition* (Eds: Taylor, T. G. & Jenkins, N. K.), 74-78. John Libbey, London.

Smart, J. L. 1993. Malnutrition, learning and behavior: 25 years on from the MIT symposium. *Proceedings of the Nutrition Society*, **52**, 189-199.

Smiseth, P. T., Örnborg, J., Andersson, S. & Amundsen, T. 2001. Is male plumage reflectance correlated with paternal care in bluethroats? *Behavioral Ecology*, **12**, 164-170.

Smith, S. M. 1991. *The black-capped chickadee: behavioral ecology and natural History*. Cornstock, Ithaca, NY.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry, third edition*. W. H. Freeman and company, New York.

Spencer, K. A., Buchanan, K. L., Goldsmith, A. R. & Catchpole, C. K. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia*

guttata). *Hormones and Behavior*, **44**, 132-139.

Stephens, P. A., Buskirk, S. W., Hayward, G. D. & Martínez del Rio, C. 2005. Information theory and hypothesis testing: a call for pluralism. *Journal of Applied Ecology*, **42**, 4-12.

Stinefelt, B., Leonard, S. S., Blemings, K. P., Shi, X. L. & Klandorf, H. 2005. Free radical scavenging, DNA protection, and inhibition of lipid peroxidation mediated by uric acid. Annals of clinical and laboratory science, **35**, 37-45.

Streif, M. & Rasa, A. E. 2001. Divorce and its consequences in the common blackbird *Turdus merula*. *Ibis*, **143**, 554-560.

Sullivan, M. S. 1994a. Discrimination among males by female zebra finches based on past as well as current phenotype. *Ethology*, **96**, 97-104.

Sullivan, M. S. 1994b. Mate choice as an information gathering process under time constraint: implications for behaviour and signal design. *Animal Behaviour*, **47**, 141-151.

Swaddle, J. P. 1996. Reproductive success and symmetry in zebra finches. *Animal Behaviour*, **51**, 203-210.

Sydeman, W. J., Pyle, P., Emslie, S. D. & McLaren, E. B. 1996. Causes and consequences of long-term partnerships in Cassin's Auklets. In: *Partnerships in birds: the study of monogamy* (ed. Black, J. M.), pp. 211-222. Oxford University Press, Oxford.

Taborsky, B. & Taborsky, M. 1999. The mating system and stability of pairs in kiwi *Apteryx* spp. *Journal of Avian Biology*, **30**, 143-151.

Tchernichovski, O., Mitra, P. P., Lints, T. & Nottebohm, F. 2001. Dynamics of the vocal imitation process: how a zebra finch learns its song. *Science*, **291**, 2564-2569.

ten Cate, C. & Mug, G. 1984. The development of mate choice in zebra finch females. *Behaviour*, **90**, 125-150.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013-1027.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and Descent of Man, 1871-1971* (Ed. by B. Campbell), pp. 136-179. Chicago, Aldine.

van Doorn, G. S. & Weissing, F. J. 2004. The evolution of female preferences for multiple indicators of quality. *American Naturalist*, **164**, 173-186.

Verrell, P. A. 1982. Male newts prefer large females as mates. *Animal Behaviour*, **30**, 1254-1255.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society, London, Series B*, **266**, 1-12.

Vos, D. R. 1995. The role of sexual imprinting for sex recognition in zebra finches: a difference between males and females. *Animal Behaviour*, **50**, 645-653.

Wagner, S. J. & Gauthreaux Jr., S. A. 1990. Correlates of dominance in intraspecific interactions of song sparrows and white-throated sparrows. *Animal Behaviour*, **39**, 522-527.

Wagner, W. E. 1998. Measuring female mating preferences. *Animal Behaviour*, **55**, 1029-1042.

Wainwright, P. E. 2002. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. *Proceedings of the Nutrition Society*, **61**, 61-69.

Ward, S., Lampe, H. M. & Slater, P. J. B. 2004. Singing is not energetically demanding for pied flycatchers, *Ficedula hypoleuca*. *Behavioral Ecology*, **15**, 477-484.

Waterland, R. A. & Garza, C. 1999. Potential mechanisms of metabolic imprinting that lead to chronic disease. *American Journal of Clinical Nutrition*, **69**, 179-197.

West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York.

Westneat, D. F., Sherman, P. W. & Morton, M. L. 1990. The ecology and evolution of extra-pair copulations in birds. In: *Current ornithology*, vol. 7, pp. 331-369 (ed. Power, D. M.). Plenum Press, New York.

Whiteman, E. A. & Côté, I. M. 2004. Monogamy in marine fishes. *Biological reviews*, **79**, 351-375.

Wickler, W. & Seibt, U. 1983. Monogamy: an ambiguous concept. In *Mate Choice* (ed. Bateson, P.), pp. 33-50. Cambridge University Press.

Widemo, F. & Sæther, S. A. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends in Ecology and Evolution*, **14**, 26-31.

Wiley, R. H. 1991. Both high- and low-ranking white-throated sparrows find novel locations of food. *Auk*, **108**, 8-15.

Williams, G. C. 1966. Natural selection, the costs of reproduction and a refinement of Lack's principle. *American Naturalist*, **100**, 687-690.

Williams, T. D. & Christians, J. K. 2003. Experimental dissociation of the effects of diet, age and breeding experience on primary reproductive effort in zebra finches Taeniopygia guttata. Journal of Avian Biology, **34**, 379-386.

Winkler, D. W. & Allen, P. E. 1996. The seasonal decline in tree swallow clutch size: physiological constraint or strategic adjustment? *Ecology*, **77**, 922-932.

Wynn, S. E. & Price, T. 1993. Male and female choice in zebra finches. *Auk*, **110**, 635-638.

Yu, B. P. 1994. Cellular defenses against damage from reactive oxygen species. *Physiological Reviews*, **74**, 139-162.

Yudkin, J. S. & Stanner, S. 1998. Prenatal exposure to famine and health in later life. *The Lancet*, **351**, 1361-1362.

Zahavi, A. 1975. Mate selection – a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205-214.

Zann, R. A. 1994. Reproduction in a zebra finch colony in south-eastern Australia: the significance of monogamy, precocial breeding and multiple broods in a highly mobile species. *Emu*, **94**, 285-299.

Zann, R. A. 1996. *The zebra finch: a synthesis of field and laboratory studies.* Oxford University Press, Oxford.